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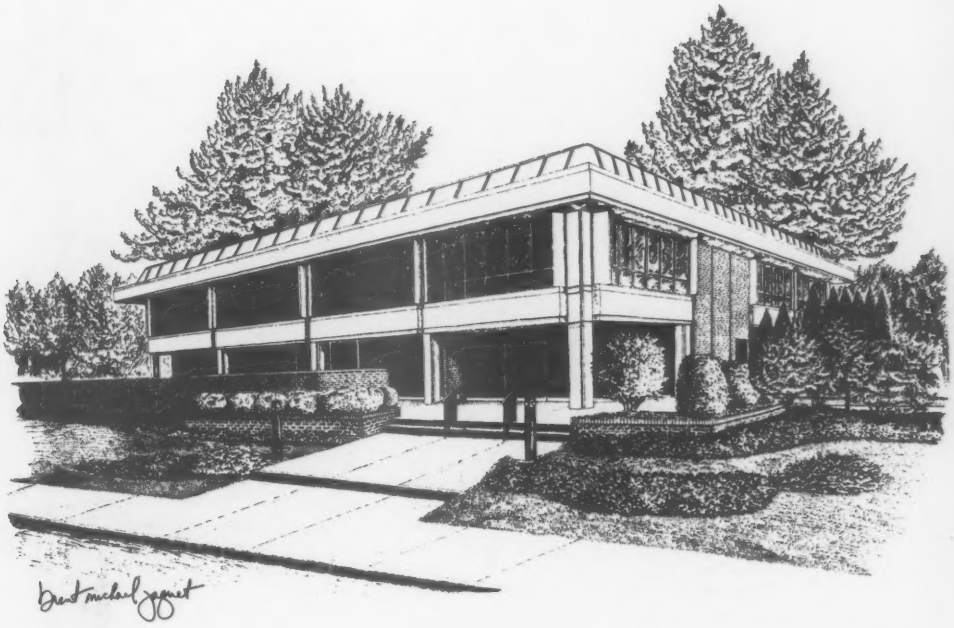

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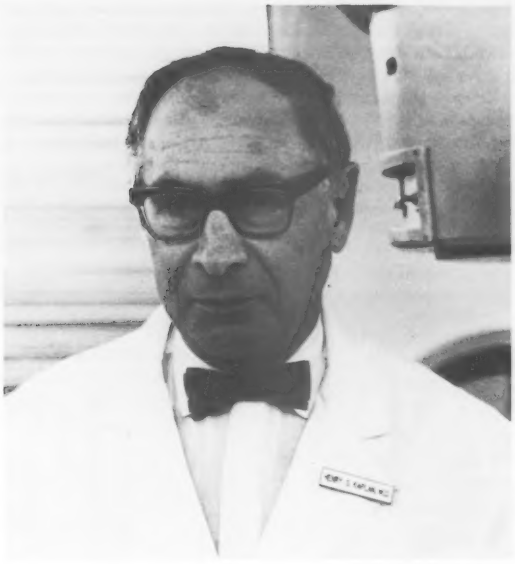

In Memoriam: HENRY SEYMOUR KAPLAN

Henry Seymour Kaplan died on 4 February, 1984. With his passing we lost a pillar of the cancer effort, but the solid foundation he did so much to build endures. Henry Kaplan was unique. He was a brilliant man and his brilliance spanned many fields: basic and clinical research, clinical medicine, teaching, and science administration. He was also a natural leader. People looked to him for guidance and he gave it willingly. In over 30 years of involvement in science and medicine, he gave unselfishly of his time and energy to keep Stanford University and the national scientific apparatus running smoothly. In the more than 20 years I knew him, he served on the National Cancer Advisory Council, the Yarbrough Commission, Boards of Scientific Counselors, and review committees too numerous to list here. In my own experience, when he was asked to do these jobs and others for the National Cancer Institute, Henry never said no. Oh, he grumbled now and then about the time spent away from home and his laboratory, but he still never said no. He was also a model of what an advisor should be, in that he possessed the one quality besides a superior intellect absolutely necessary in a great advisor: he could not deviate from the truth as he perceived it for the sake of politics or expediency, even in public. Because of this, he may have been viewed by some as a hypercritical person. He even proclaimed himself to be a "curmudgeon." But he was neither of these. He was a warm, sensitive person, blessed with a great analytical mind, who had the courage of his convictions. In short, Henry Kaplan was unique because he had greatness and he knew how to use it.

Henry Kaplan will be remembered for other things as well. His contributions to science and medicine are already history. He will be remembered for his discovery of the murine lymphoma virus; his contributions to the development of the linear accelerator; and his work in the laboratories and clinics with Hodgkin's disease and other lymphomas. To the very end he was active and productive in work on the development of human monoclonal antibodies and the molecular biology of human cancer viruses, to name but a few newer areas of interest. His colleagues in radiotherapy will remember him for establishing the sound scientific underpinning of his field of radiotherapy in the programs of the National Cancer Institute while he was a member of the National Advisory Cancer Council. I will remember him as a

good friend. A good friend who was always at his best when I needed him most, which was, for me, often enough.

I had the privilege of visiting with Henry on two occasions in the last months of his life. I told him that I had two reasons for my visits, one selfish and the other not-so-selfish. The selfish reason was to find out, for my own benefit, whether he felt all the struggles had been worthwhile. In other words, would he do it all again the same way. We recounted many shared experiences, and the insights he offered will be valuable as long as memory serves. Suffice it to say that, to the end, Henry remained Henry. The second reason was to be sure he knew how much he meant to the cancer effort. I was afraid that Henry, like people who always strive to open new frontiers, might feel he had not done enough or was not appreciated. I hope I succeeded in my second mission. He told me he was not afraid of dying. He had lived a dignified life and wanted to depart this world with dignity. In life, Henry Kaplan dignified all he touched and, in the end, he dignified dying.

Vincent T. DeVita, Jr
Director
National Cancer Institute

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691 ANNOUNCEMENTS

Relationship Between Estrogen-Receptor Proteins and Response to Chemotherapy in Breast Cancer[1]

Richard M. Levine and Marc E. Lippman[2,*]

Since the initial article in 1978 by Lippman et al (1) reporting a negative correlation between estrogen-receptor (ER) content and response to chemotherapy in patients with disseminated breast cancer, and the subsequent contradictory findings presented by Kiang et al (2), controversy involving the subject has remained active, with many other investigators publishing their observations. Some authors have reported a negative correlation between ER presence and response to chemotherapy (3-5), others have noted a positive correlation (6-9), and still others have not been able to demonstrate any relationship at all (10-12).

Several explanations for these discrepancies need to be considered. Whenever studies correlating one phenomenon with another yield a wide range of results, there is invariably an understandable tendency to assume that no relationship whatsoever exists between the variables and that the differences arose due to chance alone. On the other hand, some unrecognized difference between one study or another may provide a confounding variable which, if appreciated, might rationalize the results. This is particularly true with respect to the data under consideration, for which there are many candidates for such confounding prognostic variables. Functional ER is a necessary requirement for a hormone response. The true lack of this protein accurately predicts loss of hormone responsiveness, whereas lack of ER cannot be involved directly in chemotherapy response. Because of this, one assumes that any correlation between lack of ER and chemotherapy response will be correlative and subject to perturbations by many other prognostic variables. These variables include performance status, sites and extent of disease, prior therapy, kind of therapy, etc.

One explanation for why such disagreement exists is due in part to a variety of potential flaws in study design. The vast majority of reports are retrospective in nature and are therefore subject to several biases that can significantly influence the response rates to treatment. The studies are neither prospective nor randomized and the choice of whether or not a patient received chemotherapy, whether single-agent or combination chemotherapy was given, the duration of treatment, and the drugs and dosages employed vary considerably within each study and between studies. These decisions were probably based on each investigator's familiarity with the various chemotherapeutic agents, individual patient characteristics, and the time period (years the study reviews) that the patients were treated. All of these factors vary considerably among the studies, thus making the determination of a possible relationship between any patient characteristics (eg, tumor ER) and response to chemotherapy more difficult. For example, some investigators reserve more aggressive doxorubicin-containing regimens for certain groups of patients. Additionally, many series have only a small number of patients in each group and significant differences may be difficult to demonstrate.

Differences exist between investigators in the methods of determining ER content and in the criteria ("cut-off" level) used for determining ER positivity. Some authors chose a level as low as 3 fmols/mg of cytosol protein while others required a level of 20 fmols/mg of cytosol protein before a patient's tumor was classified as being ER-positive. It is assumed too readily that all institutions can equally perform a reliable ER determination. In fact, several recent quality assurance studies, including one conducted by the National Surgical Adjuvant Breast Project (13), have shown, unfortunately, that many ER determinations are inaccurate.

Some investigators have observed the responsiveness of distant metastases to chemotherapy and have used the ER level of the primary tumor or a previous metastatic site for analysis. Sometimes, due to the location of the metastasis or the status of the patient, a biopsy and ER determination of the metastasis are next to impossible, but it has been reported that from 15% to 38% of biopsies from multiple metastatic sites will have ER levels which differ significantly (14-16).

Whether any previous hormonal therapy or chemotherapy was administered before determining the ER level is either unknown in some reports or assumed (often incor-

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rectly) to be unchanged with intervening therapy in other reports. Allegra et al (14) have clearly shown that the level of ER can decrease dramatically after hormonal therapy. An additional selection bias is introduced by prior chemotherapy, since patients treated with any chemotherapy regimen are less responsive to subsequent treatment with any second-line chemotherapy. These two factors may dramatically influence the interpretation of a relationship between ER and response to chemotherapy but, for the most part, their significance has been ignored in the reports on the subject.

Obvious, but critical, sources of variability between reports are the criteria for determining responsiveness to chemotherapy. A wide range of subjective and objective parameters have been used by each clinician within each report and between reports, which may explain some of the differences in conclusions that have been made. Because of the controversial nature of these studies, a team of extramural medical oncologists was given the task by the National Cancer Institute of reviewing the records of patients used as the basis of results reported in studies correlating ER and chemotherapy response. In several institutions, 25% of patients were considered either nonevaluable or misclassified as to response (17).

Another aspect of the reviews which makes the determination of a relationship between ER and response to chemotherapy more confusing is that prednisone was used in combination with chemotherapy in many of the patients studied. The exact mechanism of action of prednisone in breast cancer is unknown. It may be mediated by the presence of glucocorticoid receptor, which is present in approximately 50% of human breast cancer specimens (18-20).

Furthermore, response to corticosteroids is highly correlated with the presence of ER. In one review of several studies, 28 of 83 ER-positive patients (34%) responded to glucocorticoids, whereas only one of 46 ER-negative patients responded (21). If one study (22) is excluded, in which only two of 31 patients responded overall to glucocorticoids, then the data become 26 of 59 ER-positive patients responding to glucocorticoids (44%) versus one of 39 ER-negative patients. Thus, it is clearly highly inappropriate to consider chemotherapy-containing glucocorticoids in a trial evaluating the value of ER in predicting response. Perhaps more subtly, in premenopausal women, chemotherapy may induce ovarian failure and a chemotherapy response due to such indirect means.

These comments on the problems of the previous reports evaluating ER content and responsiveness to chemotherapy may help explain why conflicting data exist and that a conclusive statement regarding the topic cannot be made with great confidence. A review of the clinical reports examining the topic has recently been published (23).

The purpose of this paper is not to critically examine the previous conflicting reports, but to present some evidence that a relationship between responsiveness to chemotherapy and absence of ER coincides with the biologic behavior of breast cancer.

Breast cancer is a tumor with a wide spectrum of behavior. Patients may present with a relatively large, slow-growing primary tumor and no distant metastases or with minimal breast involvement by tumor but with advanced, bulky metastatic disease. As long ago as 1889, it was observed that premenopausal patients with breast cancer appeared to have a worse prognosis than their postmenopausal counterparts (24) and, more recently, it has been shown that the ER status of the tumor is of prognostic importance, with ER positivity associated with a longer disease-free interval and longer overall survival (25-27). Allegra et al (28) examined the relationship between menopausal status and ER and reported that premenopausal patients tend to have a higher incidence of ER-negative primary tumors (84%) than postmenopausal patients (41%). McGuire et al (29) also found that premenopausal women more frequently had ER-negative tumors and short disease-free intervals.

Silvestri et al (30) correlated thymidine labeling index (TLI), ER status, and menopausal status. They showed that premenopausal ER-negative patients had the highest mean TLI (9.49 ± 5.28), followed by postmenopausal ER-negative patients (4.26 ± 3.93). The subgroup with the lowest TLI was postmenopausal ER-positive patients (2.64 ± 4.54). The relationship between menopausal status, the presence or absence of ER, and prognosis may be due to the different TLIs of each subgroup and the associated biologic characteristics.

One way in which the above observations may be interrelated is suggested by the following kinetic studies. Meyer and Hixon (31) reported that breast carcinomas with a high TLI appear to be associated with rapid evolution both before and after diagnosis and treatment. In 133 patients with invasive primary tumors, those who initially had local or distant spread or who developed recurrent tumor had a higher geometric mean TLI (2.93) than patients who were still free of disease (1.85) ($P < 0.05$). Women with TLIs above the 2.21 median had a significantly higher rate of early recurrence of tumor than patients with TLIs below the median ($P = 0.001$). Women < 50 years of age had a higher geometric mean TLI (2.79) than women > 50 years of age (1.96). Therefore, these data show a relationship between rapid proliferation of breast cancer with high TLIs and a younger (premenopausal?) patient population. Meyer et al (32) went on to demonstrate that ER-negative tumors have higher rates of proliferation than ER-positive tumors, which may explain the prognostic importance of ER status after primary treatment.

Schiffer et al (33) studied the kinetics of human mammary tumors as measured by TLI and its relationship to the age and positive axillary nodal status of the patient. They observed that in patients < 50 years of age, those

with axillary nodal involvement had higher TLIs than axillary node-negative patients. This was not seen in patients ⩾ 50 years of age. In women with one to three positive axillary nodes, the younger women had a statistically significant higher TLI than the older women (P = 0.004).

The relevance of the TLI is that it is a measure of the rate of DNA synthesis of the tumor and, therefore, provides information about the growth rate of the tumor. An important principle of chemotherapy is that the responsiveness of human tumors is frequently dependent on tumor growth rate (34). Anticancer drugs are more effective in rapidly growing tumors and most are cytotoxic by exerting an effect on DNA synthesis (35). Thus, the observation that ER-negative tumors have higher TLIs than ER-positive tumors should be associated with a higher responsiveness to chemotherapy.

A study which supports the proposal that ER status has potential significance in determining responsiveness to chemotherapy has been demonstrated by Kaufmann et al (36), who reported the in vitro response rate to doxorubicin and correlated it to steroid-receptor content. They reported a higher response rate in ER-negative tumors (23 responses among 38 patients) compared to ER-positive tumors (four responses among 71 patients). This was also true for the relationship of progesterone-receptor content to in vitro sensitivity. Additionally, tumors from postmenopausal patients showed more chemoresistance (66%) as compared to those of premenopausal patients (50%). Unfortunately, TLI data were not obtained.

Franco and Shafie (37) also reported increased sensitivity to doxorubicin in long-term tissue culture of ER-negative human breast cancer cells than of ER-positive cultured cells. They hypothesized that this might be due to the shorter doubling time (higher growth fraction) of the ER-negative cell line.

Livingston (38) recently reviewed the clinical literature to determine the relationship of ER and response to doxorubicin. Using a "cut-off" level of 3 fmols/mg of cytosol protein for ER positivity, he reported that among the ER-positive patients receiving predominantly non-doxorubicin-containing regimens, 71% responded, compared to 32% of ER-negative patients. For those patients who received doxorubicin-containing regimens, 50% of ER-positive and 64% of ER-negative patients responded to treatment. Therefore, a twofold increase in response rate was seen in ER-negative patients when treatment included doxorubicin compared to nondoxorubicin regimens. Clearly, such data emphasize the difficulty of retrospective comparisons between studies.

Examination of adjuvant chemotherapy trials also suggests that patients with ER-negative primary tumors are more responsive to chemotherapy than ER-positive patients. While normally stage II premenopausal ER-negative patients have a shorter relapse-free interval and overall survival than their ER-positive counterparts, when both groups (also matched for nodal status) are treated with adjuvant CMF, their 3-year relapse-free survival times are the same (35). This can be interpreted as suggesting that a greater benefit was obtained from chemotherapy in ER-negative patients compared to ER-positive patients.

These observations in the laboratory and in clinical trials support the conclusion that ER-negative patients have an increased sensitivity to doxorubicin, and that combination chemotherapy which includes this agent will result in a higher response rate than if doxorubicin is omitted.

In summary, previous clinical reports which have examined the relationship between ER content and responsiveness to chemotherapy in advanced or disseminated breast cancer are less than satisfactory. There is a multitude of critical flaws in the reviews, which are retrospective, nonrandomized, and without standardization.

The correlation of ER and the biology of breast cancer, as observed in clinical reports and in the laboratory, and studies examining tumor characteristics which influence its responsiveness to chemotherapy support the hypothesis that the presence of ER should be associated with tumors which are more well-differentiated, slower growing, and less responsive to chemotherapy. Perhaps a prospective, randomized trial is necessary to conclusively determine the relationship between ER and chemotherapy. Livingston (38) feels that the findings correlating ER negativity and increased responsiveness to doxorubicin are of potential clinical significance, and suggested a clinical trial to further study this association.

REFERENCES

1. LIPPMAN ME, ALLEGRA JC, THOMPSON EB, ET AL. The relationship between estrogen receptors and response to cytotoxic chemotherapy in metastatic breast cancer. N Engl J Med 298:1223-1228, 1978.
2. KIANG DT, FRENNING DH, GOLDMAN AI, ET AL. Estrogen receptors and responses to chemotherapy and hormonal therapy in advanced breast-cancer. N Engl J Med 299:1330-1334, 1978.
3. JANET W, and MAASS H. Some comments on the necessity of receptor determinations in human breast cancer. Cancer Res 38: 4305-4306, 1978.
4. HILF R, FELDSTEIN ML, GIBSON SL, ET AL. The relative importance of estrogen receptor analysis as a prognostic factor for recurrence or response to chemotherapy in women with breast cancer. Cancer 45:1993-2000, 1980.
5. BLAMEY RW, BISHOP HM, BLAKE JRS, ET AL. Relationship between primary breast tumor receptor status and patient survival. Cancer 46:2765-2769, 1980.
6. YOUNG PCM, EHRLICH CE, and EINHORN LH. Relationship between steroid receptors and response to endocrine therapy and cytotoxic chemotherapy in metastatic breast cancer. Cancer 46:2961-2963, 1980.
7. ROSENBAUM C, MARSLAM TA, STOLBACH LL, ET AL. Estrogen receptor status and response to chemotherapy in advanced breast cancer: the Tufts-Shattuck-Pandville experience. Cancer 46:2919-2921, 1980.
8. SAMAL BA, BROOKS SC, CUMMINGS G, ET AL. Estrogen receptors and responsiveness of advanced breast cancer to chemotherapy. Cancer 46:2925-2927, 1980.

9. MORTIMER J, REIMER R, GREENSTREET R, ET AL. Influence of estrogen receptor status on response to combination chemotherapy for recurrent breast cancer. Cancer Treat Rep 65:763-766, 1981.
10. RUBENS RD, KING RJB, SEXTAN S, ET AL. Oestrogen receptors and response to cytotoxic chemotherapy in advanced breast cancer. Cancer Chemother Pharmacol 4:43-45, 1980.
11. JANET W, MAASS H, STOLZENBACH G, ET AL. Estrogen receptor status and response to polychemotherapy in advanced breast cancer. Cancer 46:2809-2813, 1980.
12. BONNADONNA G, VALAGUSSA P, TANCINI G, ET AL. Estrogen receptor status and response to chemotherapy in early and advanced breast cancer. Cancer Chemother Pharmacol 4:37-41, 1980.
13. WITTLIFF JL, DURANT JR, and FISHER B. Methods of steroid receptor analyses and their quality control in the clinical lab. *In* Physiopathology of Endocrine Diseases and Mechanism of Hormone Action (Soto R, DeNicola AF, and Blaquier JA, eds). Alan R. Liss, Inc, New York, pp 397-411.
14. ALLEGRA J, BARLOCK A, HUFF K, ET AL. Changes in multiple or sequential estrogen receptor determinations in breast cancer. Cancer 45:792-794, 1978.
15. WEBSTER DJT, BRONN DG, and MINTON JP. Estrogen receptor levels in multiple biopsies from patients with breast cancer. Am J Surg 136:337-338, 1978.
16. KIANG DT, and KENNEDY BJ. Factors affecting estrogen receptors in breast cancer. Cancer 40:1571-1576, 1977,
17. SEARS ME, and OLSON KB. Extramural review of clinical response of breast cancer to cytotoxic chemotherapy. Cancer 46:2928-2929, 1980.
18. ALLEGRA JC, LIPPMAN ME, THOMPSON EB, ET AL. Steroid hormone receptors in human breast cancer. Proc Am Assoc Cancer Res and ASCO 19:336, 1978.
19. TEULINGS FAG, and VAN GILSE HA. Demonstration of glucocorticoid receptors in human mammary carcinoma. Horm Res 8:107-116, 1977.
20. ALLEGRA JC, LIPPMAN ME, THOMPSON EB, ET AL. Relationship between progesterone, androgen, and glucocorticoid receptor and response rate to endocrine therapy in metastatic breast cancer. Cancer Res 39:1973-1979, 1979.
21. SEIBERT K, and LIPPMAN ME. Hormone receptors in breast cancer. Clin Oncol 1:735-794, 1982.
22. RUBENS RD, and HAYWARD JL. Estrogen receptors and response to endocrine therapy and cytotoxic chemotherapy in advanced breast cancer. Cancer 46:2922-2924, 1980.
23. SINGHAKOWINTA A, SAMAL B, MARTINO S, ET AL. Is hormone sensitivity in breast cancer related to responsiveness to cytotoxic therapy? Rev Endocr-Related Cancer 11:15-19, 1982.
24. SCHINZINGER A. Veber carcinoma mammae. Verh Dtsch Ges Chir 18:28, 1889.
25. KNIGHT WA, LIVINGSTON RB, GREGORY EJ, ET AL. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. Cancer Res 37:469-4671, 1977.
26. WALT AJ, SINGHAKOWINTA A, BROOKS SC, ET AL. The surgical implications of estrophile protein estimations on carcinoma of the breast. Surgery 80:506-512, 1976.
27. LEVINE RM, and LIPPMAN ME. Breast cancer management: recent advances and recommendations. *In* Advances in Internal Medicine (Stollerman E, ed). In press.
28. ALLEGRA JC, LIPPMAN ME, SIMON R, ET AL. Association between steroid hormone receptor status and disease-free interval in breast cancer. Cancer Treat Rep 63:1271-1277, 1979.
29. Estrogen Receptors in Human Breast Cancer (McGuire WL, Carbone PP, and Vollmer EP, eds). New York, Raven Press, 1974.
30. SILVESTRI R, DAIDONE G, and DIFRONZA G. Relationship between proliferative activity and estrogen receptors in breast cancer. Cancer 44:665-670, 1979.
31. MEYER JS, and HIXON B. Advanced stage and early relapse of breast carcinomas associated with high thymidine labeling indices. Cancer Res 39:4042-4047, 1979.
32. MEYER JS, PAO BR, and STEVENS SC. Low incidence of estrogen receptor in breast carcinoma with rapid rates of cellular replication. Cancer 40:2290-2298, 1977.
33. SCHIFFER LM, BRAUNSCHWEIGER PG, STRAGANO JJ, ET AL. The cell kinetics of human mammary cancers. Cancer 43:1701-1719, 1979.
34. SHACKNEY SE, and RITCH PS. Cell kinetics. *In* Pharmacologic Principles of Cancer Treatment (Chabner BA, ed). Philadelphia, W. D. Saunders, 1982, pp 45-76.
35. DEVITA VT, JR. Principles of chemotherapy. *In* Cancer. Principles and Practice of Oncology (DeVita VT, Jr, Hellman S, and Rosenberg SA, eds). Philadelphia, J.B. Lippincott Co, 1982, pp 132-155.
36. KAUFMANN M, KLINGA K, RUNNEBAUM B, ET AL. In vitro adriamycin sensitivity test and hormonal receptors in primary breast cancer. Eur J Cancer 16:1609-1613, 1980.
37. FRANCO LA, and SHAFIE SM. Estrogen-receptor status, doubling time and sensitivity of breast carcinoma cells to adriamycin in tissue culture. Proc Am Assoc Cancer Res and ASCO 22:5, 1981.
38. LIVINGSTON RB. Breast cancer and response to chemotherapy: a possible relationship of hormone receptors and doxorubicin. Cancer Treat Rev 9:229-236, 1982.

Correlation Between Estrogen-Receptor Proteins and Response to Chemotherapy in Patients With Breast Cancer[1,2]

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The role of cytosol estrogen receptors (ER) in predicting the response of breast cancer to hormonal therapy has been well-established. Reports correlating ER status to the responses to chemotherapy, however, have generated considerable controversy. Lippman et al (1) reported that ER-negative tumors had a significantly higher response rate (75%) to chemotherapy than that of ER-positive tumors (12%), while we reported the opposite results, ie, a 36% response rate for ER-negative tumors versus an 86% response rate for ER-positive tumors (2). A consensus development meeting, "Steroid Receptors in Breast Cancer," was held to address this issue in June 1979 at the National Cancer Institute, Bethesda, MD (3).

After reviewing the collective data from 385 cases of breast cancer treated by seven investigative groups, the reviewers found that there was no difference between ER-negative and ER-positive groups in terms of their response rate to chemotherapy (3,4). However, in quantitative analysis of ER level, there appeared to be a benefit to chemotherapy in a subgroup of tumors with high ER > 50 fmols/mg (4). It should be noted that all of these studies were retrospective and had inherited certain known and unforeseen biases. We believe the differences in patient population, as studied by Lippman et al and by us, are the major factors that contributed to the contradictory results. A definitive conclusion on this issue can only be drawn from prospective studies with a uniform schedule of chemotherapeutic regimen and on a large number of patients.

Theoretically, there are many reasons to make one believe that ER-negative tumors are more sensitive to chemotherapy than ER-positive tumors. Most evidence to support this contention has been derived from the results of in vitro studies and/or through calculated deduction. For instance, in vitro studies have demonstrated that tumor cells with a high labeling index or at their proliferative phase are more susceptible to many cytotoxic chemotherapeutic agents (5,6). Since tumor cells with a high labeling index or in the proliferative phase are frequently associated with a low ER content (7,8), through logic one reaches an impression that ER-negative tumors may be apt to respond to chemotherapy in vivo. This indirect inference was further strengthened by in vitro studies showing that ER-negative tumors were more sensitive to doxorubicin than ER-positive tumors (9,10). However, Franco and Shafie (10) noted in the same study that the growth rate, not the ER status, appeared to be the determining factor in the tumor cell sensitivity to the drug.

Although correlation between tumor cell kinetics and sensitivity to chemotherapeutic agents is well-documented by in vitro studies, this concept is not necessarily transferable to the in vivo clinical situation. Most in vitro studies do not take into consideration factors such as the heterogeneity of tumor cell populations, the limitation of host toxicity, and the in vivo kinetics of drug metabolism. In assessing the role of cell kinetics in cancer chemotherapy, Valeriote and Edelstein (11) stated: "Cell kinetics can be used as a framework to both predict and explain the cellular actions of anticancer drugs. It cannot stand alone, and it is not a unique solution to the problems of cancer chemotherapy." After critically reviewing the literature, Tannock (12) found that pretreatment indices of tumor cell kinetics are of little value in predicting response. This conclusion is also shared by other investigators (13,14).

Clinical data are also not in accordance with the results from these in vitro studies. Following chemotherapy, one would observe an increase of ER level should chemotherapy mainly suppress the ER-negative cells as shown in the in vitro studies. Rather, most reports have indicated that chemotherapy does not alter the tumor ER content (15-17). Furthermore, should chemotherapy mainly suppress the ER-negative cell population, one would expect an increased response rate by utilizing a combination modality of chemotherapy and hormonal therapy, since hormonal therapy is known to affect mainly the ER-positive tumor cells. Again, most combination trials of chemotherapy and hormonal therapy in the gen-

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eral breast cancer population failed to improve the response rate (18–21). These negative results suggest that chemotherapy is probably effective for both ER-positive and ER-negative cells.

It is also not unusual that an opposite result to an in vitro study may occur in the clinical setting. ER-negative tumors with a rapid growth rate act as a double-edged sword: they may have a favorable response to chemotherapy, but this response can be quickly nullified by their inherent disadvantages, such as a rapid tumor regrowth and genetic instability. Association of hormonal independency with a high incidence of aneuploidy has been observed in both human and mouse mammary tumors (22,23). These disadvantages may explain why human breast cancer with a high labeling index or of ER-negative nature is frequently associated with a poor prognosis (24–26).

An objective clinical response of human breast cancer can be accomplished not only by the administration of effective drugs but also by numerous other factors. One of these factors is the growth rate of tumor cells. Unless the volume of cell killing from repeated courses of chemotherapy is greater than the regrowth of surviving tumor cells, remission cannot be achieved or sustained. Therefore, the rapid regrowth rate in an ER-negative tumor can attenuate the response rate as well as the duration of response. Of those studies reporting a similar response rate to chemotherapy between ER-negative and ER-positive breast cancers, the duration of remission is frequently noted to be longer for ER-positive than for ER-negative tumors (27,28), suggesting that the slow regrowth rate could be one of the favorable factors rendering a longer duration of remission in patients with ER-positive tumors.

Flares of breast cancer during the initiation of hormonal therapy have been documented in human and animal breast cancers. Both animal and in vitro cell culture studies have demonstrated that low-dose estrogen may stimulate the tumor growth and enhance the tumor sensitivity to cytotoxic agents, while high-dose estrogen suppresses this sensitivity (29,30). An attempt to improve the response rate to chemotherapy by estrogen stimulation has been pursued by Lippman et al (31) and by Allegra et al (32). This illustrates that the sensitivity to chemotherapy of an ER-positive tumor can be *varied* according to the host environment.

It seems that the real issue is not whether ER directly relates to the chemotherapeutic response. As we suggested earlier (2), some undefined ER-associated factor(s) may be more intimately involved in determining the results of chemotherapy. Such a factor(s) may be the determinant to many peripheral functions, such as the response to chemotherapy and the level of ER. To establish a relationship between the two peripheral functions may not always be successful, and may often be quite difficult.

REFERENCES

1. LIPPMAN ME, ALLEGRA JC, THOMPSON EB, ET AL. The relation between estrogen receptors and response rate to cytotoxic chemotherapy in metastatic breast cancer. N Engl J Med 298:1223-1228, 1978.
2. KIANG DT, FRENNING DH, GOLDMAN AI, ET AL. Estrogen receptors and responses to chemotherapy and hormonal therapy in advanced breast cancer. N Engl J Med 299:1330-1334, 1978.
3. DE SOMBRE ER, CARBONE PP, JENSEN EV, ET AL. Special Report: Steroid Receptors in Breast Cancer. N Engl J Med 301:1011-1012, 1979.
4. COLE DK, SEARS ME, and OLSON KB. The relationship of quantitative estrogen receptor level and clinical response to cytotoxic chemotherapy in advanced breast cancer: an extramural analysis. (Personal communication, manuscript submitted for publication.)
5. SKIPPER HE. Kinetics of mammary tumor cell growth and implications for therapy. Cancer 28:1479-1499, 1971.
6. DREWINKO B, PATCHEN M, YANG LY, ET AL. Differential killing efficacy of twenty antitumor drugs on proliferating and nonproliferating human tumor cells. Cancer Res 41:2328-2333, 1981.
7. MEYER JS, RAO BR, STEVENS SC, ET AL. Low incidence of estrogen receptor in breast carcinomas with rapid rates of cellular replication. Cancer 40:2290-2298, 1977.
8. JAKEZ R, SMITH CA, AITKIN S, ET AL. Influence of cell proliferation and cell cycle phase on expression of estrogen receptor in MCF-7 cells. Proc Am Assoc Cancer Res 24:180, 1983.
9. KAUFMANN M, KLINGA K, RUNNEBAUM B, ET AL. In vitro adriamycin sensitivity test and hormonal receptors in primary breast cancer. Eur J Cancer 16:1609-1613, 1980.
10. FRANCO LA, and SHAFIE SM. Estrogen receptor status, doubling time and sensitivity of breast carcinoma cells to adriamycin in tissue culture. Proc Am Assoc Cancer Res 22:5, 1981.
11. VALERIOTE FA, and EDELSTEIN MB. The role of cell kinetics in cancer chemotherapy. Semin Oncol 4:217-226, 1977.
12. TANNOCK I. Cell kinetics and chemotherapy: a critical review. Cancer Treat Rep 62:1117-1133, 1978.
13. HALL TC. Limited role of cell kinetics in clinical cancer chemotherapy. *In* Prediction of Response in Cancer Therapy (Hall TC, ed). Natl Cancer Inst Monogr 34:15, 1971.
14. BUSH H. The relevance of cell kinetics for human cancer, Eur J Cancer (suppl 1):213-218, 1980.
15. KIANG DT, and KENNEDY BJ. Factors affecting estrogen receptors in breast cancer. Cancer 40:1571-1576, 1977.
16. ALLEGRA JC, BARLOCK A, HUFF KK, ET AL. Changes in multiple or sequential estrogen receptor determinations in breast cancer. Cancer 45:792-794, 1980.
17. MOBBS BG. Effect of time and therapy on the hormone receptor status of breast carcinomas. Can Med Assoc J 127:217-221, 1982.
18. BRUNNER KW, SONNTAG RW, ALBERTO P, ET AL. Combined chemo- and hormonal therapy in advanced breast cancer. Cancer 39:2923-2933, 1977.
19. TORRI S, HOIROYDE C, BRODOVSKY H, ET AL. Tamoxifen plus sequential CMF chemotherapy versus tamoxifen alone in post-menopausal patients with advanced breast cancer: a randomized trial. Cancer 45:111-117, 1979.
20. RUBENS RD, BEGENT RHJ, KNIGHT RK, ET AL. Combined cytotoxic and progestogen therapy for advanced breast cancer. Cancer 42:1680-1686, 1978.
21. CAVALLI F, BEER M, MARTZ G, ET AL. Concurrent or sequential use of cytotoxic chemotherapy and hormone treatment in advanced breast cancer: report of the Swiss Group for Clinical Cancer Research. Br Med J 1:5-8, 1983.
22. KUTE TE, MUSS HB, ANDERSON D, ET AL. Relationship of steroid receptor, cell kinetics and clinical status in patients with breast cancer. Cancer Res 41:3524-3529, 1981.
23. KIANG DT, KING M, ZHANG H-J, ET AL. Cyclic biological expression in mouse mammary tumors. Science 216:68-70, 1982.

24. STRAUS MJ, and MORAN, RE. The cell cycle kinetics of human breast cancer. Cancer 46:2634-2639, 1980.
25. STEWART JF, KING RJ, SEXTON SA, ET AL. Oestrogen receptors, sites of metastatic disease and survival in recurrent breast cancer. Eur J Cancer 17:449-453, 1981.
26. KIANG DT, FRENNING DH, GAY J, ET AL. Combination therapy of hormone and cytotoxic agents in advanced breast cancer. Cancer 47:452-456, 1981.
27. RUBENS RD, KING RJ, SEXTON S, ET AL. Oestrogen receptors and response to cytotoxic chemotherapy in advanced breast cancer. Cancer Chemother Pharmacol 4:43-45, 1980.
28. COCCONI G, DELISI V, MORI P, ET AL. Estrogen receptors and response to chemotherapy in advanced breast cancer. Tumori 68:67-71, 1982.
29. MARKAVERICH BM, MEDINA D, AND CLARK JH. Effects of combination estrogen: cyclophosphamide treatment on the growth of the MXT transplantable mammary tumor in the mouse. Cancer Res 43:3208-3211, 1983.
30. WEICHSELBAUM R, HELLMAN S, PIRO A, ET AL. Proliferation kinetics of a human breast cancer line in vitro following treatment with 17β-estradiol and 1-β-D-arabinofuranosylcytosine. Cancer Res 38:2339-2342, 1978.
31. LIPPMAN ME, CASSIDY J, WESLEY M, ET AL. A randomized attempt to increase the efficacy of cytotoxic chemotherapy in metastatic breast cancer by hormonal synchronization. Proc ASCO 1:79, 1982.
32. ALLEGRA JC, WOODCOCK TM, RICHMAN SP, ET AL. A phase II trial of tamoxifen, premarin, methotrexate and 5-fluorouracil in metastatic breast cancer. Breast Cancer Res Treat 2:93-99, 1982.

Alternating Pulses of Vincristine-Prednisone With Cytarabine-Cyclophosphamide Versus Vincristine-Prednisone in the Maintenance Therapy of Acute Lymphoblastic Leukemia[1,2,3]

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From January 1976 to December 1978, 347 children ⩽ 15 years of age were entered in a collaborative controlled trial which included: induction (vincristine-daunorubicin-prednisone); intensification (cytarabine-cyclophosphamide); CNS prevention (intrathecal methotrexate-dexamethasone, three doses during induction and three weekly doses during the first month of maintenance, followed by one dose every 3 months for 48 months); and maintenance (6-mercaptopurine daily and methotrexate twice weekly with reinforcement pulse doses of either 1.5 mg/m² × 1 of vincristine plus 40 mg/m²/day × 7 of prednisone [*Arm A*] or vincristine-prednisone alternating with 50 mg/m² of cytarabine sc every 12 hours × 10 plus 600 mg/m² × 1 of cyclophosphamide [*Arm B*]). Pulses were performed in both arms at 1, 2, 3, 4, and 6 months and every 3 months thereafter. Randomization was stratified according to age and initial wbc count. A total of 89% (310/347) of patients achieved complete remission. Duration of continuous complete remission was evaluated according to prognostic factor groups. At 5 years, 34.5% of patients with good prognosis, 24.8% with intermediate prognosis, and 12.8% with poor prognosis are in continuous complete remission. There is statistical difference between good versus poor prognosis ($P < 0.0005$) and intermediate versus poor prognosis ($P < 0.025$). Moreover, 5-year survival is 50.9%, 35.2%, and 18.2% in the good-, intermediate-, and poor-prognosis groups, respectively. Duration of continuous complete remission up to the first event (ie, bone marrow, CNS, or other extramedullary relapse, or death in complete remission), according to prognostic groups, did not differ in relation to reinforcement pulses (Arm A or B). We conclude that there was no benefit in alternating pulses of vincristine-prednisone with cyclophosphamide-cytarabine as used in this study. [Cancer Treat Rep 68:581–586, 1984]

Over the past 20 years, there has been an increase in the proportion of patients with acute lymphoblastic leukemia (ALL) who achieve long-term, disease-free survival. Despite the therapeutic improvements, > 50% of the patients eventually relapse and die. The major obstacle to cure continues to be hematologic relapse, which is usually observed during the maintenance phase of treatment. The aim of the maintenance phase of therapy is the con-

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tinuous reduction of leukemic cells, and combinations of drugs during remission are clearly superior to single agents in preventing relapse.

In January 1976, the Grupo Argentino de Tratamiento de la Leucemia Aguda (GATLA) started a controlled clinical trial for the treatment of patients with newly diagnosed ALL. The main objective of this study was to test if more aggressive reinforcement pulses are more effective in the maintenance therapy of this disease.

MATERIALS AND METHODS

A total of 347 children ≤ 15 years of age with previously untreated ALL were entered on GATLA protocol 1-LLA-76 from January 1976 to December 1978. The present evaluation is based on findings through April 1983. The range of the follow-up period is from 52 to 88 months, with a median of 70 months.

Patients were considered off-study if they were lost to follow-up or had bone marrow relapse (> 25% lymphoblasts in the bone marrow aspirate), CNS relapse, testis relapse, or death in complete remission. Bone marrow aspiration and lumbar puncture for cytological study of the cerebrospinal fluid were performed at diagnosis, on Days 14 and 28 of the induction period, and every 3 months thereafter for 48 months. Therapy protocol was as follows.

Induction.—Children with an initial wbc count < 20,000/mm^3 received vincristine (1.5 mg/m^2 iv weekly) and prednisone (40 mg/m^2 orally daily); if the bone marrow was not M1 by Day 29, daunorubicin (30 mg/m^2 iv weekly) was added to the regimen. Children with an initial wbc count > 20,000/mm^3 received weekly vincristine-daunorubicin and daily prednisone (at the same dosages) from the outset.

Intensification.—All patients received cytarabine (50 mg/m^2 sc every 12 hours × 10) plus cyclophosphamide (600 mg/m^2 iv × 1 on the first day of this treatment phase).

CNS prevention.—All patients were given intrathecal methotrexate (12 mg/m^2/dose) plus dexamethasone (4 mg/m^2/dose), three doses during induction (Days 1, 14, and 28) and three weekly doses during the first month of maintenance followed by one dose every 3 months thereafter for 48 months.

Maintenance.—All patients received 6-mercaptopurine (90 mg/m^2 orally daily) and methotrexate (15 mg/m^2 orally or im twice weekly) for 48 months. Patients were randomized between *Arm A*, which received vincristine (1.5 mg/m^2 iv × 1) and prednisone (40 mg/m^2 orally for 7 days), and *Arm B*, which received the same courses of vincristine-prednisone alternating with cytarabine (50 mg/m^2 sc every 12 hours × 10) and cyclophosphamide (600 mg/m^2 iv × 1 on the first day). Reinforcement pulses were performed in both arms at 1, 2, 3, 4, and 6 months and every 3 months thereafter. During reinforcement pulses with cytarabine plus cyclophosphamide (Arm B), maintenance was discontinued for 7 days according to hematologic tolerance. In addition, patients were randomized to receive or not to receive levamisole (120 mg/m^2 orally daily) as adjuvant immunotherapy.

The randomization for both variables was done prospectively by stratification according to initial wbc count (< and > 20,000/mm^3). Life table methods (1) were employed to obtain the curves, and the log rank test (2) was used to compare the incidence of relapse among curves. A chi-square test was used to test the difference between proportions.

To evaluate the results of induction and complete remission duration, the following prognostic group criteria were adopted (3): *good prognosis*—wbc count < 10,000/mm^3 and 2–8 years of age at diagnosis; *intermediate prognosis*—wbc count between 10,000 and 50,000/mm^3 and any age, or wbc count < 10,000/mm^3 and age < 2 years or > 8 years; *poor prognosis*—wbc count > 50,000/mm^3 and any age.

In relation to CNS preventive method, the results of this study can be compared to our previous studies, which used cranial irradiation and intrathecal methotrexate-dexamethasone (4) and the administration or not of levamisole as adjuvant immunotherapy (5).

RESULTS

Of the 347 children entered in this study, 310 (89%) achieved complete remission. Thirty-seven children were removed from the study during the induction period because of partial remission (eight patients), no remission (ten), or death (19). The results of induction treatment according to prognostic group are shown in table 1. Ninety-six percent (113/118) of the good-prognosis group, 88% (141/160) of the intermediate-prognosis group, and 81% (56/69) of the poor-prognosis group achieved complete remission. There is a statistical difference in the response rate between groups: good versus intermediate, $P < 0.05$; and good versus poor, $P < 0.005$. The first event according to prognostic factor groups is shown in table 2. Analyzing these data, we can observe a statistical difference ($P < 0.01$) between the number of children with CNS relapse in the good versus poor prognosis groups, and also in the number of children who continue in complete remission in the good versus poor prognosis groups.

The actuarial curve up to the first event and survival according to the prognostic groups adopted in this study are depicted in figures 1 and 2, respectively. At 5 years, 34.5% of the good-prognosis, 24.8% of the intermediate-prognosis, and 12.8% of the poor-prognosis groups are in continuous complete remission. There is a statistical difference between good versus poor prognosis ($P < 0.0005$) and intermediate versus poor prognosis ($P < 0.025$). In relation to survival, we can observe that at 5 years,

TABLE 1.—Results of induction treatment, according to prognostic group*

Prognostic group	No. of patients (%)				
	Complete response	Partial response	No response	Died	Total
Good	113 (96)	—	—	5	118
Intermediate	141 (88)	6	4	9	160
Poor	56 (81)	2	6	5	69
Total	310 (89)	8	10	19	347

*Good vs intermediate, $P < 0.05$; good vs poor, $P < 0.005$.

TABLE 2.—First event, according to prognostic factor group

	Prognostic group*			
	Good	Intermediate	Poor	Total*
Total achieving complete remission	113	141	56	310
Relapsed				
Bone marrow	28 (25)	49 (35)	20 (36)	97 (31)
CNS	18 (16)†	24 (17)	19 (34)†	61 (20)
Testes	6 (5)	5 (3)	1 (2)	12 (4)
CNS + testes	1 (1)	— (—)	— (—)	1 (0.3)
Eye	1 (1)	— (—)	— (—)	1 (0.3)
Bone marrow + CNS	— (—)	1 (1)	1 (2)	2 (0.6)
Bone marrow + testes	— (—)	— (—)	1 (2)	1 (0.3)
Died in continuous complete remission	15 (13)	16 (11)	3 (5)	34 (11)
Lost to follow-up	8 (7)	14 (10)	4 (7)	26 (8)
Continue in continuous complete remission	36 (32)†	32 (23)	7 (12)†	75 (24)

*Values = No. of patients (%).
†$P < 0.01$.

50.9% of the children with good prognosis, 35.2% with intermediate prognosis, and 18.2% with poor prognosis are alive. There is a significant statistical difference between good versus intermediate ($P < 0.005$), good versus poor ($P < 0.0005$), and intermediate versus poor ($P < 0.005$) prognosis.

Duration of continuous complete remission up to the first event (ie, bone marrow, CNS, or any other extramedullary relapse, or death in complete remission), according to the reinforcement pulses evaluated in this study, is shown in figures 3-5 for good-, intermediate-, and poor-prognosis groups, respectively. Four patients in the intermediate-prognosis group and three in the poor-prognosis group were excluded from evaluation of duration of complete remission because of CNS leukemia infiltration at diagnosis. As can be observed, there was no difference in results from vincristine-prednisone (Arm A) or vincristine-prednisone alternating with cyclophosphamide-cytarabine (Arm B) pulses in any of the prognostic groups.

Eleven percent (34/310) of the children died in complete remission: 8.7% (14/161) and 14.1% (20/142) from Arms A and B, respectively. This difference is not significant. The causes of death for Arm A were varicella (three patients), sepsis (five), and pneumonitis (six); five patients belonged to a good-prognosis group and nine belonged to an intermediate-prognosis group. The causes of death for Arm B were sepsis (eight patients), pneumonitis (four), enteritis (two), hemorrhage (two), hepatitis (one), viral infection (one), and unknown (two); six patients belonged to good-, ten belonged to intermediate-, and four belonged to poor-prognosis groups.

In general, the therapeutic regimens used in this study were well-tolerated and were not associated with severe toxicity. The mean nadir wbc counts 7-15 days after the pulses were the following: Arm A—2160/mm^3 (range, 700-4800); and Arm B—1853/mm^3 (range, 200-4100).

DISCUSSION

Over the past 15 years, attempts have been made to improve therapy for patients with ALL, with an increasing rate of cure. Since remission rates have been 85%-95% with currently used induction therapy, successful induction remission is no longer an essential problem. However, one-half of the patients will eventually relapse

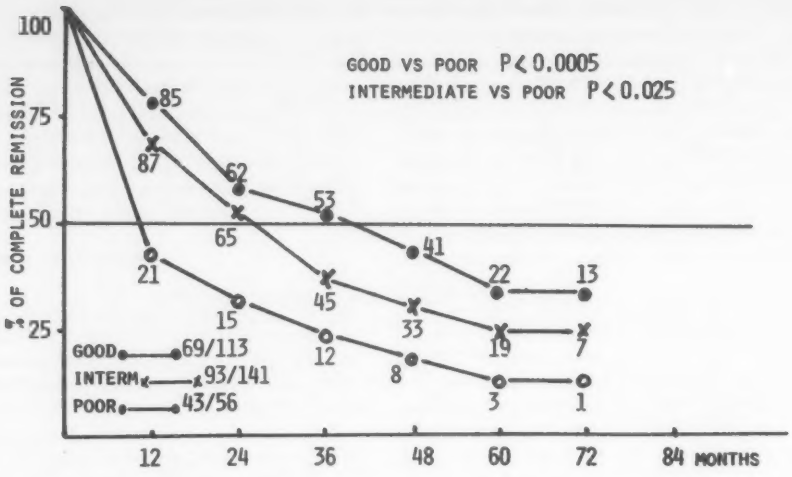

FIGURE 1.—Duration of continuous complete remission up to the 1st event, by prognostic group.

(most of them during maintenance phase). Therefore, maintenance therapy is the phase in most need of improvement, because most therapeutic failures are due to bone marrow relapse, ie, the failure of systemic therapy to prevent emergence of resistant leukemic cells.

Of the available agents used in the maintenance phase, 6-mercaptopurine and methotrexate are the most effective (6). Several authors have suggested that "pulses" of induction regimen drugs should be given periodically throughout maintenance phase. The rationale of regimens in which pulses of additive therapy are given during maintenance was that drugs having different mechanisms of action would further reduce the residual leukemic cell population.

Our data show that the addition of alternating pulses of cyclophosphamide-cytarabine to vincristine-prednisone, as used in this study, does not offer positive evidence of effectiveness in prolonging the period of continuous complete remission in any of the prognostic groups adopted in this study. However, it is necessary to recog-

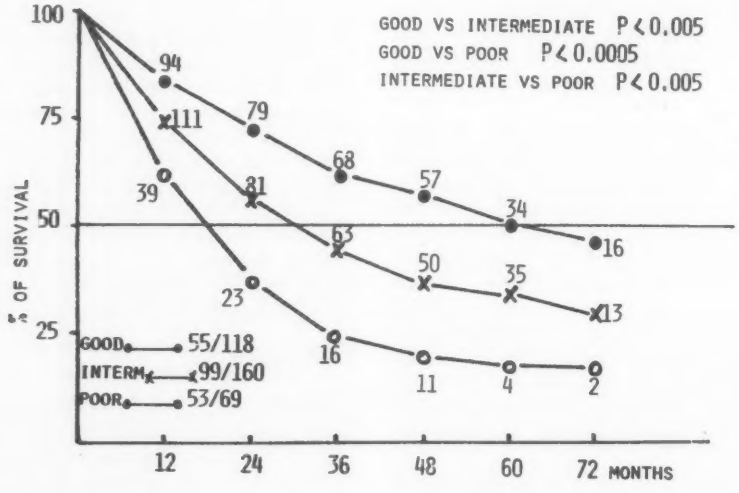

FIGURE 2.—Duration of survival, according to prognostic group.

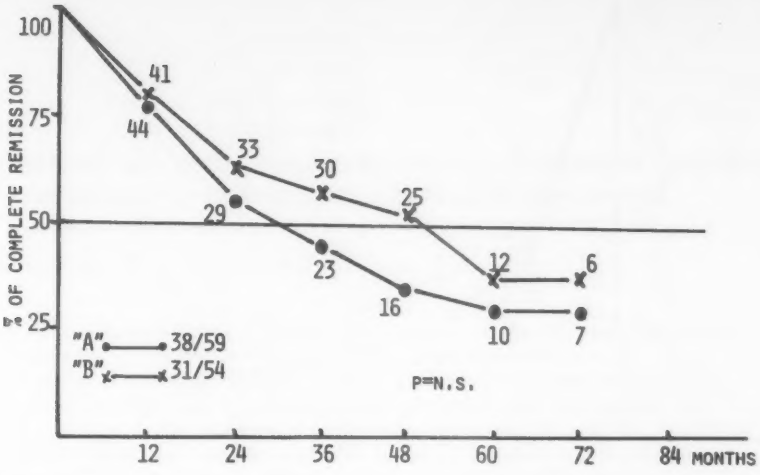

FIGURE 3.—Duration of continuous complete remission up to the 1st event in good-prognosis group, according to reinforcement pulses.

nize that cyclophosphamide-cytarabine added to vincristine-prednisone is more toxic, judging from the number of deaths in complete remission and the nadir wbc count immediately after the pulses.

A possible explanation of the inefficacy of alternating pulses would be that all groups still need a more aggressive therapy if we want to improve the results. In this sense, the Berlin protocol, employing more aggressive and prolonged induction and intensification regimens, offers a good example (7). Obviously, the treatment in one phase of therapy can influence the outcome in another phase, although many times it is difficult to obtain direct evidence of the benefit of each because they are interrelated. In this sense, it is interesting to remember an early study of the Childrens Cancer Study Group, which showed that the additive therapy is beneficial in those patients with the most favorable prognosis (8). However, an early Southwest Oncology Group study, which did not include CNS prevention, showed that vincristine added to prednisone for reinforcement after induction of remission with vincristine-prednisone did not increase the duration of hematologic remission or survival over that achieved with prednisone reinforcement alone (9). Nevertheless, in comparative studies, the results have indi-

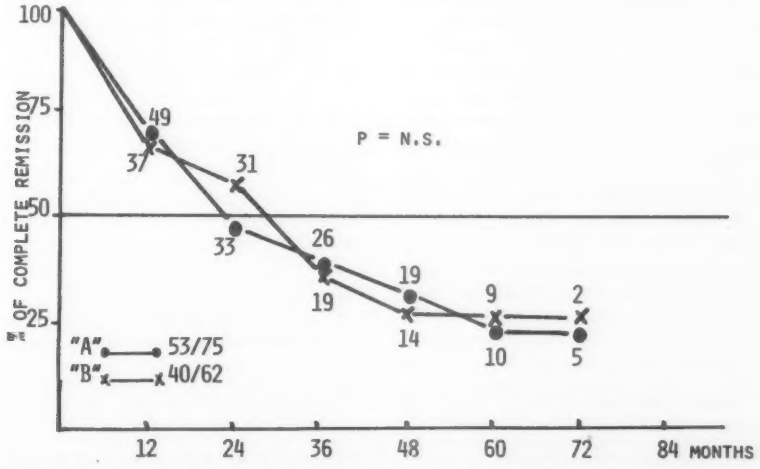

FIGURE 4.—Duration of continuous complete remission up to the 1st event in intermediate-prognosis group, according to reinforcement pulses.

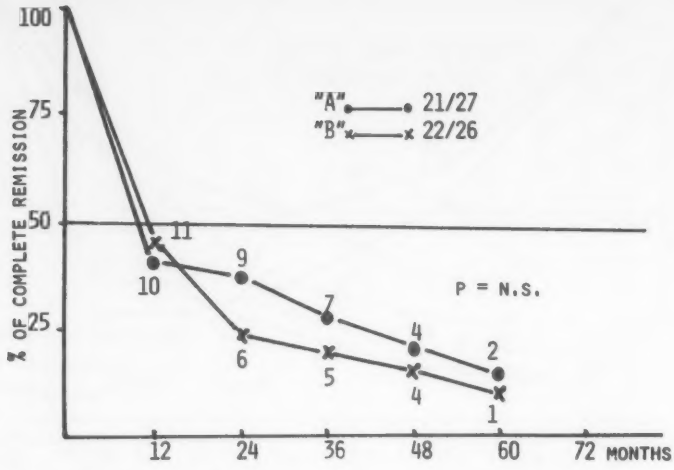

FIGURE 5.—Duration of continuous complete remission up to the 1st event in poor-prognosis group, according to reinforcement pulses.

cated that with an adequate induction regimen, CNS prevention, and effective maintenance therapy, "pulses" do not make a significant contribution to the overall results (10,11).

A very recent report, which analyzes the prognostic factors and therapy for ALL, emphasizes the importance and usefulness of clinical prognostic factors in identifying subsets of children at low and high probability of treatment failure (12). Also, it has been demonstrated that children with good prognosis can have reduced therapy without adversely affecting CNS relapse rate, bone marrow relapse, or survival (13).

As a final comment on our poor overall results in relation to duration of continuous complete remission and survival, the above-mentioned study (12) showed a continuous complete remission at 4 years (by newly identified prognostic factors) of 79.7%, 58.6%, and 43.1% for good-, average-, and poor-prognosis groups, respectively. We believe that several factors adversely affected our results. For instance, we administered neither L-asparaginase nor cranial irradiation, which are known to contribute to improvement in results for ALL. Finally, low socioeconomic class with poor income surely affects the overall results of this collaborative clinical trial.

We conclude that there is no benefit in alternating pulses of vincristine-prednisone with cytarabine as used in this study.

REFERENCES

1. CUTLER SJ, and EDERER F. Maximum utilization of the life table method in analyzing survival. J Chronic Dis 8:699-712, 1958.
2. PETO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J Cancer 35:2-39, 1977.
3. MILLER DR, LEIKIN S, ALBO V, ET AL. Use of prognostic factors in improving the design and efficiency of clinical trials in childhood leukemia: Children's Cancer Study Group report. Cancer Treat Rep 64:381-392, 1980.
4. SACKMANN MURIEL F, SVARCH E, PAVLOVSKY S, ET AL. Comparison of central nervous system prophylaxis with cranial radiation and intrathecal methotrexate vs intrathecal methotrexate alone in acute lymphoblastic leukemia. Blood 62:241-250, 1983.
5. PAVLOVSKY S, SACKMANN MURIEL F, GARAY G, ET AL. Chemoimmunotherapy with levamisole in acute lymphoblastic leukemia. Cancer 48:1500-1507, 1981.
6. AUR RJA, SIMONE JV, VERZOSA MS, ET AL. Childhood acute lymphocytic leukemia: Study VIII. Cancer 42:2123-2134, 1978.
7. RIEHM H, GADNER H, HENZE G, ET AL. The Berlin childhood acute lymphoblastic leukemia therapy study, 1970-1976. Am J Pediatr Hematol Oncol 2:299-306, 1980.
8. MILLER DR, SONLEY M, KARON M, ET AL. Additive therapy in the maintenance of remission in acute lymphoblastic leukemia of childhood: the effect of the initial leukocyte count. Cancer 34:508-517, 1974.
9. FERNBACH DJ, GEORGE SL, SUTOW WW, ET AL. Long term results of reinforcement therapy in children with acute leukemia. Cancer 36:1552-1559, 1975.
10. AUR RJA, HUSTU HO, VERZOSA MS, ET AL. Comparison of two methods of preventing central nervous system leukemia. Blood 42:349-357, 1973.
11. ORTEGA JJ, and JAVIER G. Evaluation of reinduction in a modern protocol for the treatment of childhood acute lymphoblastic leukemia. Analysis of the D-74 protocol. Sangre (Barc) 26:47-57, 1981.
12. MILLER DR, LEIKIN S, ALBO V, ET AL. Prognostic factors and therapy in acute lymphoblastic leukemia of childhood: CCG-141. A report from Children's Cancer Study Group. Cancer 51:1041-1049, 1983.
13. COCCIA PF, BLEYER WA, SIEGEL SE, ET AL. Reduced therapy for children with good prognosis acute lymphoblastic leukemia (ALL). Blood 58 (Suppl 1):137a, 1981.

High-Dose Methotrexate With Leucovorin Rescue, Vinblastine, and Bleomycin With or Without Tamoxifen in Metastatic Renal Cell Carcinoma[1]

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A regimen of high-dose methotrexate with leucovorin rescue, vinblastine, and bleomycin with or without tamoxifen was administered to 34 patients with metastatic renal cell carcinoma. No complete remissions were observed, but ten patients (30%) achieved partial remission and an additional 13 patients (39%) had stabilization of disease. The median survival of responding patients (110 weeks) was significantly longer than that of nonresponding patients. The addition of tamoxifen did not influence response or survival. This regimen was tolerated without significant toxicity. A prospective randomized study of the vinblastine and high-dose methotrexate regimen compared to the respective single agents seem to be indicated. [Cancer Treat Rep 68:587–590, 1984]

Metastatic renal cell carcinoma (RCC) has been shown to be generally resistant to all chemotherapeutic agents evaluated to date. Few studies have shown response rates > 20% for single agents (1,2). Consequently, efforts to improve the response rate using conventional chemotherapeutic agents have involved combination chemotherapy regimens.

The rationale for the present study was based on several factors. Vinblastine had demonstrated moderate activity against RCC, with a 25% response rate among 135 patients (3,4). No data were available on the activity of methotrexate (1), but in view of reports of an expanded spectrum of activity in solid tumors with high-dose methotrexate and leucovorin rescue (5) and experimental evidence that a vinca alkaloid may enhance the cytotoxicity of this agent when given 60–80 minutes before methotrexate (6), the present schedule was derived. Furthermore, preliminary evidence had suggested some efficacy for bleomycin in RCC (7), and previous studies had demonstrated cell synchrony and increased effect when bleomycin was administered 6 hours after the vinca alkaloid (8). Thus, the final kinetically based schedule of vinblastine, high-dose methotrexate, and bleomycin was determined for this study.

Hormonal therapy, particularly with progesterone derivatives, has produced some responsiveness in RCC (9). Bloom et al (10) reported an inhibitory effect of an antiestrogen compound on estrogen-induced RCC in hamsters and suggested a potential therapeutic benefit in man. Tamoxifen, at a dose comparable to that used in another hormone-responsive cancer (ie, breast), was therefore added to the therapeutic regimen in this study after an initial consecutive series of patients received the combination chemotherapy alone.

PATIENTS AND METHODS

From April 1977 to January 1980, 34 patients with metastatic RCC were entered in this trial. All patients had a histologically confirmed diagnosis of RCC, with at least one area of measurable disease. In addition, all patients had a clinically estimated survival of > 2 months, no serious intercurrent disease, and adequate renal and bone marrow functions (serum creatinine < 0.13 mmol/L, wbc count > 4 × 10^9/L, and platelet count > 100 × 10^9/L). Patients who had had prior chemotherapy, excluding progesterone derivatives, were not eligible for the study. All patients were followed until death.

Each patient had full staging of disease extent before treatment. A clinical examination, with measurement of tumors in two dimensions, was performed; cbc, liver function tests, blood urea, serum creatinine, creatinine clearance, chest x-ray, and abdominal ultrasound were obtained. Whole-lung tomograms and radionuclide liver and bone scans were obtained if clinically indicated. Prior to each course of chemotherapy, cbc, serum creatinine, and creatinine clearance were repeated. Chest x-ray and other imaging procedures were repeated at 2-month in-

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TABLE 1.—Patient characteristics*

Characteristic	Chemotherapy alone (n = 19)	Chemotherapy + tamoxifen (n = 15)	All patients (n = 34)
Sex			
Male	11	7	18
Female	8	8	16
Median age in yrs (range)	59 (22-71)	59 (23-67)	58 (22-71)
Performance status†			
0	0	0	0
1	2	3	5
2	11	6	17
3	4	5	9
4	2	1	3
Prior therapy			
None	1	1	2
Nephrectomy	14	13	27
Radiotherapy with or without nephrectomy	3	1	4
Progesterone	1	0	1
Predominant site of disease			
Lung	12	6	18
Liver	0	2	2
Bone	3	4	7
Lymph nodes	4	3	7

*Unless specified otherwise, values = No. of patients.

†Eastern Cooperative Oncology Group (ECOG) criteria: 0 = fully active; 1 = ambulatory, capable of light work; 2 = in bed < 50% of the time, capable of self-care but not of work; 3 = in bed > 50% of the time, capable of only limited self-care; and 4 = completely bedridden.

tervals to assess response.

All patients received a kinetically based schedule of vinblastine (4 mg/m^2) given iv at 0 hour, methotrexate (500 mg/m^2) given by a 4-hour iv infusion starting at 1 hour, and bleomycin (30 mg) given im at 6 hours. At 29 hours patients began to receive leucovorin (15 mg) orally every 6 hours for 12 doses. Courses of vinblastine, methotrexate, and leucovorin were repeated at 14-day intervals. Bleomycin was given weekly, to a maximum dose of 360 mg. The initial dose of vinblastine was increased to 6 mg/m^2 if bone marrow tolerance was adequate, and the methotrexate dose was increased by increments of 500 mg/m^2/course to a maximum of 2 g/m^2 if tolerance permitted. After the initial 19 consecutive patients had received this regimen, tamoxifen (40 mg/day orally) was added for the subsequent 15 patients. Patients who showed definite evidence of tumor progression were removed from the study. Patients showing objective regression or stabilization of disease continued to receive therapy until there was clear-cut evidence of disease progression or relapse. Complete remission (CR) was defined as the disappearance of all clinically detectable tumor, as determined both by physical examination and by imaging procedures. Partial remission (PR) was defined as a decrease in size by at least one-half of all measurable disease, with no new lesions. Stable disease was defined as < 50% regression of measurable disease, with no new lesions and no deterioration in performance status. Progressive disease was defined as an increase in size of preexisting measurable disease or the appearance of new lesions.

Response rates were compared using the chi-square test of contingency tables. Survival and duration of response were calculated using the life-table method and were compared using the log-rank test (11).

RESULTS

Thirty-four patients with metastatic RCC were entered in the study. Nineteen patients received combination chemotherapy alone and 15 patients received combination chemotherapy plus tamoxifen. The characteristics of both patient groups are shown in table 1. One patient was lost to follow-up and was therefore not evaluable.

There were no significant differences in the response rate between the two groups. No CRs were observed; five patients (36%) receiving chemotherapy plus tamoxifen and five patients (26%) receiving chemotherapy alone achieved PR, for an overall response rate of 30%. A additional four patients (28%) and nine patients (47%) in the respective groups had stable disease. The best responses were seen in patients with good pretreatment performance status (ECOG 0-2) and predominant lymph node involvement. Nine patients (41%) with a good pretreatment performance status achieved PR compared to only one patient (10%) with a poor performance status (ECOG

3,4). PRs were observed in four of seven patients (57%) with predominant lymph node involvement, while five of 18 patients (28%) with predominant lung, one of six (17%) with predominant bone, and none of two with predominant liver involvement achieved PR. Four patients in the chemotherapy-alone group received subsequent progesterone therapy, without benefit, when relapse or disease progression occurred; among the 14 patients who received secondary chemotherapy only one short-time response was observed (with lomustine).

The median duration of response for patients achieving PR was 57 weeks compared to 18 weeks for patients with stable disease ($P = 0.001$); there was no significant difference between the two treatment groups. There was a significantly better survival for patients achieving PR when compared to patients with stable and progressive disease (fig 1). The median survival for all patients with PR was 110 weeks compared to 32 weeks for patients with stable disease and 10 weeks for patients with progressive disease ($P = 0.001$). Considering initial performance status, PR patients with good performance status (ECOG 1,2) had a median survival of 109 weeks, while patients with stable and progressive disease with a similar initial performance status had a median survival of 29 weeks ($P < 0.005$). There were also significantly better median survival times for PR patients compared to the remainder with predominant lymph node (113 vs 50 weeks) and lung (75 vs 19 weeks) involvement. No significant differences in survival were observed between those patients receiving chemotherapy plus tamoxifen and those receiving chemotherapy alone.

The toxicity of this chemotherapy regimen has not been severe, with mild to moderate skin and mucosal effects occurring in 21 patients and mild to moderate nausea and vomiting occurring in 21 patients. Hematological toxicity was minimal, with < 5% of the courses resulting in significant leukopenia ($< 3 \times 10^9$ cells/L); thrombocytopenia ($< 100 \times 10^9$ cells/L) was even less common (1%). The most severe toxic effects were a life-threatening pulmonary reaction which developed in one patient and a severe peripheral neuropathy which occurred in another. Mild renal dysfunction (serum creatinine, 0.15–0.2 mmol/L) occurred in only two patients. Apart from one patient experiencing moderate flushing with tamoxifen, no toxic effects were observed with this agent added to the regimen.

DISCUSSION

The results observed in this study raise several points of interest. The 30% overall response rate achieved is higher than that usually reported for single agents, but considering the relatively small numbers of patients in the study and the 95% confidence limits for the true response rate (18%–55%), it is not clear that this regimen is superior. Nevertheless, a survival advantage has been demonstrated for responding patients even when taking into account initial performance status and sites of disease involvement. Baumgartner et al (12) have recently reported an improved survival for responding patients receiving a high-dose methotrexate with leucovorin program. Their results in conjunction with the present data

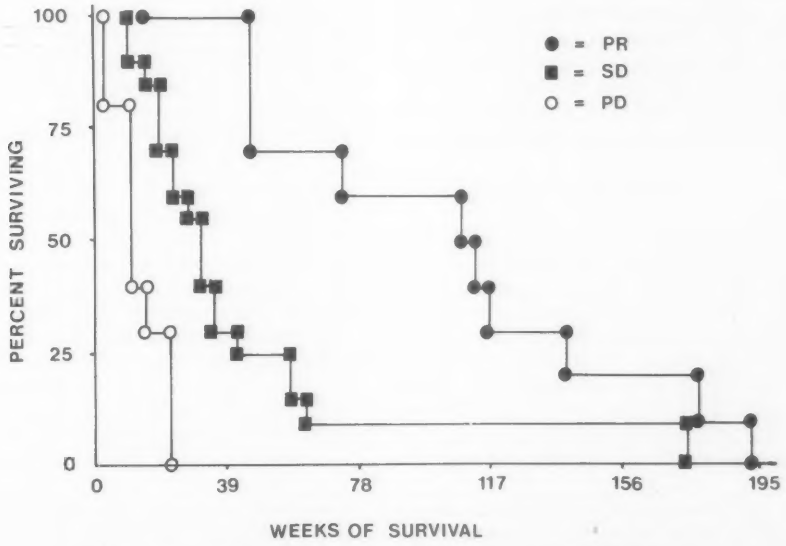

FIGURE 1.—Survival of all patients according to remission status (log-rank analysis $P < 0.001$).

support further evaluation of this approach.

In the present study, patients with an initially good performance status and predominant lymph node or lung involvement had the best outcome, which has been noted in other studies (13). This may partly be a reflection of the ease of measurement of disease in lymph nodes and lung compared to bone and liver, but also probably indicates that the overall bulk of disease at presentation is less in patients with predominant lymph node or lung involvement.

Very little significant toxicity occurred in our study. In particular, there was virtually no renal toxicity, despite the high-dose methotrexate and the fact that most of the patients had had a nephrectomy. This is in contrast to the study of Baumgartner et al (12) who reported considerable toxicity. All of our patients received the methotrexate as inpatients, and meticulous attention was paid to initial hydration, alkalinization of urine, and urinary output over the 24-hour posttreatment period. With this attention to detail, it is possible to deliver high doses of methotrexate safely to this high-risk patient population.

The addition of tamoxifen to this regimen of combination chemotherapy added neither a response nor a survival advantage; considering the low response rate recently reported for this agent in RCC (13), it seems unlikely that continued combined use of this hormone with chemotherapy will confer significant benefit. Bleomycin has now been shown in other studies to be of little therapeutic value in RCC, either as a single agent or in combination chemotherapy (14,15). It is also likely in the present study that bleomycin added little to the combination of methotrexate and vinblastine. While the current results are at the very most only suggestive of improved efficacy for the combination of methotrexate and vinblastine over single-agent therapy, in a disease with such a poor prognosis these results deserve further attention. It would therefore seem reasonable to consider a randomized evaluation of the combination of methotrexate and vinblastine compared to each agent individually, to define the role of this approach in the treatment of metastatic RCC.

REFERENCES

1. CARTER SK, and WASSERMAN TH. The chemotherapy of urologic cancer. Cancer 36:729-747, 1975.
2. LOKICH JJ, and HARRISON JH. Renal cell carcinoma: natural history and chemotherapeutic experience. J Urol 114:371-374, 1975.
3. HAGAN V, TRAPP JD, RHAMY RV, ET AL. Treatment of metastatic renal cell carcinoma. South Med J 67:1175-1178, 1974.
4. HRUSHESKY WJ, and MURPHY GP. Current status of the therapy of advanced renal carcinoma. J Surg Oncol 9:277-288, 1977.
5. BLEYER WA. The clinical pharmacology of methotrexate. Cancer 41:36-51, 1978.
6. BENDER RA. Membrane transport of methotrexate (NSC-740) in human neoplastic cells. Cancer Chemother Rep Part 3, vol 6(1):73-82, 1975.
7. BLUM RH, CARTER SK, and AGRE KA. A clinical review of bleomycin—a new antineoplastic agent. Cancer 31:903-914, 1973.
8. LIVINGSTON RB, BODEY GP, GOTTLIEB JA, ET AL. Kinetic scheduling of vincristine (NSC-67574) and bleomycin (NSC-125066) in patients with lung cancer and other malignant tumors. Cancer Chemother Rep 57:219-244, 1973.
9. MORALES A, KIRULTA G, and HOLT G. Hormones in the treatment of metastatic renal cancer. J Urol 114:692-693, 1975.
10. BLOOM HJG, LOE FJC, and MITCHLEY BCV. Sex hormones and renal neoplasia. Inhibition of tumor and hamster kidney by an estrogen antagonist, an agent of possible therapeutic value in man. Cancer 20:2118-2124, 1976.
11. PETO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 35:1-39, 1977.
12. BAUMGARTNER G, HEINZ R, ARBES H, ET AL. Methotrexate-citrovorum factor used alone and in combination chemotherapy for advanced hypernephromas. Cancer Treat Rep 64:41-46, 1980.
13. AL-SARRAF M, EYRE H, BONNET J, ET AL. Study of tamoxifen in metastatic renal cell carcinoma and the influence of certain prognostic factors: a Southwest Oncology Group study. Cancer Treat Rep 65:477-451, 1981.
14. RICHARDS F, MUSS HB, WHITE DR, ET AL. CCNU, bleomycin and methylprednisolone with or without adriamycin in renal cell carcinoma: a randomized trial. Cancer Treat Rep 61:1591-1593, 1977.
15. HAHN DM, SCHIMPFF SC, RUCKDESCHEL JC, ET AL. Single-agent therapy for renal cell carcinoma: CCNU, vinblastine, thioTEPA, or bleomycin. Cancer Treat Rep 61.1585-1587, 1977.

Combined Modality Approach in the Management of Locally Advanced Head and Neck Cancer[1]

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Twenty-five patients with stage III or IV locally advanced head and neck cancer were treated from February 1981 to November 1982 with a combined modality approach utilizing chemotherapy followed by irradiation and surgery. Induction therapy with cisplatin and bleomycin was followed by a sequence integrating weekly cisplatin into a program of locoregional irradiation. After the administration of 4800 cGy, patients received either "boost" irradiation or surgery. At the completion of all therapy, 20 of 23 patients evaluable for response had achieved complete remission. Toxicity was tolerable and all patients are being followed closely to assess durability of remission and overall survival. [Cancer Treat Rep 68:591–597, 1984]

Despite major advances in surgical and radiation therapy techniques, the control rate for locally advanced carcinoma of the head and neck (large primary tumors; T_3, T_4), advanced nodal disease (N_2, N_3), or both, remains poor. The 2-year survival rate is only 15%–30% and disease often recurs locally in the head and neck region after single modality therapy (1). The factors that influence survival of patients with locally advanced squamous cell carcinoma of the head and neck are stage of disease at presentation, location of the primary tumor, degree of tumor differentiation, type or types of therapy, performance status, and the institution in which they are being treated (2).

Treatment plans combining radiation therapy and surgery for the management of advanced local and regional disease have resulted in better local control of the tumor (3). However, survival after combined therapy has been no better than survival after either modality used alone (4). Until recently, chemotherapy has been relegated to a palliative role to be used for the patient with recurrent disease after initial therapy with surgery and/or irradiation. Several trials utilizing chemotherapy followed by irradiation or surgery have shown significant tumor response but no improvement in overall survival (5–8).

For the past 2½ years, we have been utilizing a closely integrated combined modality approach with initial chemotherapy for the treatment of these advanced cancers. Our approach has been based on the following underlying principles: (*a*) that the drugs utilized, cisplatin and bleomycin, have a high initial response rate in advanced head and neck cancers (9,10); (*b*) that cisplatin may have a dual role, functioning both as an active chemotherapeutic agent and as a radiation potentiator (11–14); and (*c*) that the duration of initial response to the chemotherapeutic agents themselves may be brief (15). This phase I–II study was specifically designed to closely integrate an intense chemotherapy program into an irradiation sequence to take advantage of cisplatin's dual role as an active chemotherapeutic agent and as a radiation potentiator. This paper addresses the question of whether or not this sequence can give a high response rate with acceptable toxicity.

MATERIALS AND METHODS

Each patient with advanced local head and neck cancer was evaluated by a multimodality group prior to the initiation of therapy. Surgical evaluation included biopsy and staging of the lesion, tattoo of the margins for potential surgical resection, and panendoscopy under general anesthesia to evaluate for multiple sites of disease. The operability of a patient was determined prior to any therapeutic intervention. A medical evaluation was obtained to determine the acceptability for vigorous chemotherapy. The metastatic evaluation

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consisted of a history and physical examination, cbc, liver function tests, chest x-ray, and optional bone scan. Renal function studies (BUN, creatinine, and 24-hour urinary creatinine clearance), pulmonary function studies (FEV_1 and vital capacity), and audiometry were also obtained. Radiation therapy evaluation included detailed treatment planning with construction of the appropriate treatment positioning devices. Complete eligibility requirements were as follows: locally advanced disease (stage III or IV), no evidence of distant metastases, evaluable lesions, no previous treatment by chemotherapy or irradiation to the involved area, life expectancy of > 6 weeks, Eastern Cooperative Oncology Group performance status of 3 or better, BUN ≤ 20 mg/100 ml, creatinine ≤ 1.5 mg/100 ml, creatinine clearance > 60 ml/min, initial wbc count ≥ 4000/mm^3, platelet count ≥ 100,000/mm^3, pulmonary function tests with vital capacity ≥ 60% of normal, PAO_2 ≥ 70 mm Hg on room air, panendoscopy, chest x-ray, and informed consent.

In this study there were 19 males and six females. The mean age was 62 years, with a range of 33–86. Six patients had stage III disease and 19 had stage IV disease. Primary sites included: nasopharynx in one patient; oral cavity in two; oropharynx in nine; hypopharynx in seven; larynx in one; paranasal sinus in four; and auricle in one.

The treatment phase of this protocol included admission to the hospital for the administration of chemotherapy. On Day 1 of the treatment phase, the patient received 100 mg/m^2 of cisplatin by rapid iv administration after the appropriate premedication. On Days 2–5, a continuous iv bleomycin infusion was initiated (15 units/m^2 administered over 24 hours). On completion of the 4-day bleomycin infusion, the patient was discharged. On Day 21, the patient was re-admitted for a second rapid iv administration of cisplatin utilizing 100 mg/m^2. The cisplatin dose was reduced to 50% if creatinine was between 1.5 and 2.0 mg/100 ml, if the wbc count was < 3000/mm^3, or if the platelet count was < 100,000/mm^3. Cisplatin was not given if creatinine was > 2.0 mg/100 ml, if the wbc count was < 2000/mm^3, or if the platelet count was < 50,000/mm^3. There were no specific dose modifications for bleomycin.

On Day 22, the patient's previously arranged radiation therapy was initiated. Radiation was administered to a total 4800-cGy tumor dose in 180–200-cGy daily increments five times per week for 5 weeks, to include both the primary site with adequate margins and both sides of the neck. Therapy was administered utilizing a Varian 4-MeV linear accelerator with the appropriate field size and positioning devices. Each field was treated each day.

Beginning on Day 28, the patient received cisplatin at a dose of 20 mg/m^2 iv once per week during the initial 5 weeks of radiation therapy. For the first 15 patients entered in this trial, the cisplatin was administered at a dose of 20 mg/m^2 three times per week. When possible, this was administered on an outpatient basis. One hour after this infusion the patient's radiation therapy was administered.

At completion of 4800 cGy to both the primary tumor site and neck fields, the patient was given a 2-week rest and then re-evaluated by the multimodality team. If the patient had initially been considered to be operable and had agreed to surgery, a surgical resection was performed. If the patient was initially considered to be inoperable, had refused surgery, or was felt to be too great an operative risk, a boost of 2000 additional cGy was administered to the primary tumor site and to the site of any previously diagnosed neck disease. This was administered with 180–200 cGy fractions/day but without any subsequent cisplatin administration. The patient was evaluated for tumor response at the completion of this consolidation therapy and followed at 1–2-month intervals by the multimodality team. This protocol was specifically designed to evaluate patient response after chemotherapy, at 4800 cGy, and at the completion of all therapy.

Response Evaluation

Complete response (CR) was defined as the apparent complete disappearance of all measurable and palpable disease and the normalization of x-rays. Partial response (PR) was defined as tumor shrinkage of all measurable disease by > 50% of the product of the perpendicular of the two largest dimensions of each lesion. Minor response was defined as measurable tumor shrinkage but not enough to satisfy the criterion for a PR. No change or stable disease was defined as tumor measurements that did not change during the course of therapy. Progression of disease was defined as tumor measurements that increased during treatment.

Response evaluation was specifically done at three points. The initial response to chemotherapy alone was evaluated on Day 22, just after the second large dose of inpatient cisplatin. The second measurement was done 2 weeks after the completion of 4800 cGy, and a third was obtained at the completion of all therapy, including boost irradiation or surgery. Pathologic response was evaluable for those patients who underwent surgical resection.

RESULTS

There were 25 patients entered in this protocol (table 1); all were evaluable for toxic effects. Of these patients, 23 were also evaluable for response to therapy. The remaining patients had had surgery prior to the initiation of chemotherapy and irradiation. Eighteen patients had some shrinkage of tumor with chemother-

TABLE 1.—Patient characteristics

Patient No.	Primary site	Stage	Tumor grade	Cisplatin (mg/m^2)	% maximun calculated dose	Status at—*		
						Day 22	4800 cGy	End of therapy
1	Oropharynx, left	$T_3N_2M_0$	II	380	76	MR	PR	CR
2	Floor of mouth, left	$T_3N_2M_0$	I	100	20	PR	CR	CR
3	Tonsil, left	$T_3N_1M_0$	III	222	44	MR	CR	CR
4	Nasopharynx	$T_4N_2M_0$	II	340	68	NR	PR	PR
5	Antrum, right	$T_4N_0M_0$	III	170	34	NR	NR	NR
6	Hypopharynx, left	$T_4N_0M_0$	II	349	70	MR	PR	CR
7	Multiple	$T_4N_2M_0$	II	294	59	NR	PR	PR
8	Larynx	$T_4N_1M_0$	II	394	79	MR	CR	CR
9	Maxilla, left	$T_4N_2M_0$	III	301	60	PR	CR	CR
10	Auricle	$T_4N_0M_0$	II	323	65	PR	PR	CR
11	Antrum, right	$T_4N_1M_0$	III	361	72	MR	PR	CR
12	Hypopharynx	$T_3N_0M_0$	III	247	49	NR	CR	CR
13	Base of tongue	$T_3N_1M_0$	II	271	54	MR	PR	CR
14	Hypopharynx	$T_3N_2M_0$	III	250	50	PR	CR	CR
15	Base of tongue	$T_3N_1M_0$	II	260	52	PR	PR	CR
16	Retromolar trigone, right	$T_4N_2M_0$	II	323	79	PR	CR	CR
17	Sphenoid sinus	$T_4N_0M_0$	III	148	49	I	I	I
18	Hypopharynx	$T_4N_1M_0$	III	167	56	MR	PR	CR
19	Tonsil, left	$T_4N_2M_0$	II	200	67	MR	CR	CR
20	Retromolar trigone, right	$T_3N_1M_0$	III	283	94	PR	CR	CR
21	Hypopharynx	$T_3N_1M_0$	I	257	86	NR	CR	CR
22	Alveolar ridge	$T_3N_0M_0$	II	280	93	MR	CR	CR
23	Hypopharynx	$T_3N_2M_0$	II	138	46	PR	CR	CR
24	Hypopharynx	$T_3N_0M_0$	II	262	87	PR	CR	CR
25	Base of tongue	$T_3N_2M_0$	I	249	83	MR	CR	CR

*MR = minor response; NR = no change or disease stabilization; I = inevaluable.

apy alone, but by Day 22 no patient had shrinkage that satisfied the requirements for a CR. After the initial 4800 cGy had been delivered, nine of 23 patients were considered to have a PR, 13 were considered to have a clinical CR, one patient was a nonresponder, and two patients were inevaluable due to prior surgery. One patient with a clinical PR died of a suspected cardiac arrhythmia 7 months after therapy. He had been considered a clinical partial responder to chemotherapy and irradiation because his computerized tomographic scan showed incomplete resolution of a mass in the nasopharynx. At postmortem examination this patient had no pathologic evidence of disease. Four of the 15 patients not treated by subsequent surgical resection but with full-dose radiation therapy only had biopsy-negative primary tumor sites at some time after the completion of therapy. These biopsy specimens were obtained between 3 and 6 months after therapy. Eight patients had a surgical resection after receiving initial irradiation. Four of these patients had residual tumor in the surgical specimen after receiving 4800 cGy. All surgical margins were clear and the residual tumor usually consisted of microscopic residual disease only. Four patients had no tumor seen microscopically after 4800 cGy and adequate surgical resection.

The total dose of cisplatin administered averaged 259 mg/m^2. The mean inpatient dose of cisplatin was 182 mg/m^2, with a range of 97–234 mg/m^2. The mean outpatient cisplatin dose administered was 77 mg/m^2, with a range of 0–185 mg/m^2. This represents 64% of the total calculated cisplatin dose for each patient. Our initial schema had attempted to utilize cisplatin on an outpatient basis at a dose of 20 mg/m^2 three times per week during the course of radiation therapy. This was difficult to administer because it met with poor patient compliance and severe toxicity. During this interval two patients developed wbc counts < $2000/mm^3$. Most patients had substantial nausea and concomitant

weight loss during this period. The protocol was subsequently revised after the first 15 patients to include cisplatin administration once per week at a dose of 20 mg/m^2 on an outpatient basis. The patient compliance at this dose was much more acceptable. There was no discernible difference in either overall response rate or durability of response with the change from three times per week to one time per week.

It is important to note that Patient 10 had an invasive squamous cell carcinoma of the auricular area. This tumor biologically did not have a clinical course consistent with a skin cancer. This was a much more aggressive tumor and the patient was placed on the protocol to be evaluated for both response and toxicity. It was felt that this patient's prognosis was otherwise very poor and that all attempts should be made to try to gain local control of the disease. Patient 17 had a squamous cell carcinoma of the sphenoid sinus that had microscopic residual disease at the resection margins. This patient could not be evaluated for response but could be evaluated for both toxicity and overall survival. Patient 21 had a left neck dissection prior to the initiation of therapy. The primary site had not been identified prior to surgery but the patient was still evaluable for response at the primary site as well as for toxicity. A further breakdown of response data is shown in table 2.

Toxicity

Toxicity from this intensive treatment program was substantial though tolerable (table 3). All toxic effects were graded according to the standard Radiation Therapy Oncology Group scoring criteria for acute radiation morbidity on a scale of 0-IV. Twelve of 25 patients required interruption of their radiation treatment sequence due to mucositis: three patients developed grade I; eight, grade II; one, grade III; and 0, grade IV. This usually occurred during the third week of therapy and corresponded to a dose between 2200 and 2800 cGy. The duration of this interruption was between 1 and 2 weeks. Mucositis was felt to be a consequence of these patients' poor nutritional status, the dose rate of irradiation, and the large radiation treatment field sizes. The role of cisplatin in causing earlier or more severe mucositis than expected was not clear. This mucositis was readily reversible with treatment interruption. Subsequent resumption of irradiation proved to be well-tolerated. Three patients developed some high-tone hearing loss during their cisplatin administration. In one patient this hearing loss was felt to be due to disease progression. The other two patients had ototoxicity thought to be cisplatin-induced. There was no clear correlation between the total dose of cisplatin received and this side effect. Twelve patients had a > 10-pound weight loss during the course of therapy. This proved to be a very difficult problem that was only partially remedied by more aggressive nutritional support. Seven patients developed a wbc count depression $\leq 2000/mm^3$ during their treatment. This was usually seen at the end of the third week of therapy with low-dose cisplatin and irradiation, and corresponded to a radiation dose between 2200 and 2800 cGy. A concomitant decrease in the hemoglobin and platelet count occurred in only one patient. This patient developed an infection around the tracheostomy and died of sepsis with disseminated intravascular coagulation (DIC). No pulmonary toxicity was noted. One patient developed increased BUN and creatinine, which were believed to be due to dehydration and proved to be reversible with aggressive iv fluid support. All patients had some degree of cisplatin-induced nausea and vomiting. This was moderately well-controlled with an antiemetic combination of dexamethasone and metaclopramide. Six of the patients who underwent composite resections after chemotherapy and irradiation to a dose of 4800 cGy had some delay of wound healing. The cause for this is unclear and is undergoing intensive investigation.

DISCUSSION

This protocol was designed to closely integrate a chemotherapy sequence into a radiation therapy/surgery program. The combination of cisplatin and bleomycin was chosen because these agents have been shown by previous investigators to have a high degree of activity in the treatment of advanced carcinomas of the head and neck (9-17). Methotrexate was specifically eliminated from this sequence because of the belief that it might in-

TABLE 2.—Response*

	CR	PR	MR	NR	Inevaluable
Chemotherapy only (Day 22)	0	9	10	5	1
Chemotherapy + initial radiation therapy (4800 cGy)	14	9	0	1	1
End of all therapy	21	2	0	1	1

*Values = No. of patients.

TABLE 3.—Toxicity

Patient No.	Toxic effect (grade)	Dose (cGy)	Duration (wks)	Surgery	Current status	Comments
1	—	—	—	+	NED*	28 mos
2	—	—	—	-	Dead	Only 5000 cGy, 22 mos, arrhythmia, local control
3	Mucositis (II); ototoxicity (III)	2600	2	-	Dead	Only 6000 cGy, 21 mos, local failure
4	Ototoxicity (II)	—	—	-	Dead	Arrhythmia, 7 mos, postmortem NED
5	Ototoxicity (II)	—	—	-	Dead	Refused cisplatin, 5 mos, local and metastatic disease
6	Weight loss (II)	—	—	-	Dead	24 mos, primary controlled, 2nd primary, lung
7	Mucositis (II)	2800	2	-	Dead	6.5 mos, primary controlled, lung metastases
8	Weight loss (II); wbc count, 1000/mm^3; died with DIC	—	—	-	Dead	2 mos, DIC from tracheostomy infection
9	Weight loss (II)	—	—	-	NED	20 mos
10	Weight loss (II)	—	—	-	NED	20 mos
11	—	—	—	-	Dead	17 mos, local and metastatic disease
12	Weight loss (II); mucositis (II)	1800	2	-	NED	19 mos
13	Weight loss (II); mucositis (II)	2800	2	+	Dead	12 mos, local failure
14	Mucositis (I)	2200	1	-	Dead	11 mos, local failure
15	Mucositis (II)	2600	2	-	NED	18 mos
16	Weight loss (II); mucositis (I)	2800	1	+	NED	15 mos, primary negative, node microscopically positive
17	Weight loss (II)	—	—	+	NED	14 mos
18	Weight loss (II); mucositis (III)	2200	2	+	NED	14 mos, neck dissection negative
19	Weight loss (II); mucositis (II); wbc count, 1900/mm^3	2400	2	-	Dead	9 mos, primary controlled, metastatic disease
20	Weight loss (II)	—	—	-	NED	13 mos
21	Weight loss (II); wbc count, 2000/mm^3	—	—	+	NED	13 mos
22	Mucositis (II); wbc count, 1900/mm^3; creatinine, 2.4 mg/100 ml	2800	2	+	NED	13 mos, surgical specimen negative
23	Mucositis (I); wbc count, 1900/mm^3	—	—	-	Dead	12 mos, suicide, postmortem negative
24	Mucositis (II); wbc count, 2800/mm^3	3200	1	-	NED	13 mos
25	Mucositis (I); wbc count, 1800/mm^3	2200	1	+	Dead	13 mos, wound complications, postmortem negative

*NED = no evidence of disease.

ease oral toxicity. Cisplatin was utilized to take advantage of its double role as an active anticancer agent (18-22) and as a radiation potentiator (11-14,23,24). It was felt that the cisplatin should be given as close to the irradiation as possible and in as high a dose as could be tolerated for this radiation potentiation to take effect. As the protocol evolved, it became clear that platinum was difficult to administer on an outpatient basis three times per week. Patient tolerance and acceptance were poor. A once per week outpatient cisplatin administration was empirically utilized after the 15th patient. Data from the University of Minnesota corroborated the tolerability of once per week administration of cisplatin at a dose of 20 mg/m^2 (25).

Utilization of this schema does require very close patient monitoring during therapy. Significant mucositis and weight loss occurred in many patients during treatment. Though difficult to quantify, the mucositis has become relatively predictable and usually occurs at a dose level between 2200 and 2800 cGy. This corresponds to the second to third outpatient dose of cisplatin. To rectify this problem, we have initiated a 1-week interruption in the radiation sequence at the 2000-cGy point. This 1-week break after the second week of irradiation may also help with the problem that we have noticed of an apparent mild cumulative bone marrow toxicity and suppression of the wbc count. Weekly cisplatin administration may not give the bone marrow sufficient time to recuperate from the cumulative effects of this agent. We have not seen a significant problem with platelet count depression. A second method to alleviate some of this toxicity will be to decrease our radiation dose rate from 200 to 180 cGy/treatment. Finally, the nutritional consequences of this protocol have been substantial. There is a subjective impression that the nutritional depletion from the combined modality approach may well be enhanced over that seen with either chemotherapy or irradiation alone. The nutritional consequences of radiation therapy as a single modality in advanced head and neck cancers have been well-described (26).

Although these are preliminary data, all 25 patients have been followed for > 1 year. The average follow-up is 16 months, with a range of 13-29 months. To date, 12 of 25 patients are still alive without disease. Three patients have died with uncontrolled local disease only, at 21, 11, and 10 months after therapy. Two patients have died of uncontrolled local and metastatic disease 5 and 17 months after therapy. Two patients have died of metastatic disease only, at 4 and 12 months after therapy. One patient has died of a second primary (lung), three have died of treatment-related causes (one with disseminated intravascular coagulation from a wound infection 1 month after initial radiotherapy and two with postoperative wound problems 9 and 12 months postoperatively, with no evidence of disease at postmortem examination), and two have died of unrelated causes (both of myocardial infarctions 7 and 22 months after therapy). Adequate local control has been attained in 20 of 25 patients with advanced local head and neck cancer. We will continue to closely follow these patients to see whether this protocol makes an impact upon survival at 5 years.

REFERENCES

1. GLICK JG, and MARCIAL V. The adjuvant treatment of inoperable stage III and IV epidermoid carcinoma of the head and neck with platinum and bleomycin infusions prior to definitive radiotherapy. Cancer 46:1919-1926, 1980.
2. MILLION RR, CASSISI NJ, and WITTES RD. Cancer in the head and neck. *In* Cancer: Principles and Practice of Oncology (DeVita VT, Hellman S, and Rosenberg SA, eds). Philadelphia, J. B. Lippincott, 1982, Chap 13, pp 301-395.
3. FLETCHER GH. Textbook of Radiotherapy. Philadelphia, Lea and Febiger, 1980.
4. CACHIN Y, and ESCHWEGE F. Combination of radiotherapy and surgery in the treatment of head and neck cancer. Cancer Treat Rev 2:177-191, 1975.
5. VOGL SE, LERNER H, KAPLAN BH, ET AL. Failure of effective initial chemotherapy to modify the course of stage IV (M0) squamous cancer of the head and neck. Cancer 50:840-844, 1982.
6. KNOWLTON AH, PERCESPLO B, BOBROWS, ET AL. Methotrexate and radiation therapy in the treatment of advanced head and neck tumors. Radiology 116:709-712, 1975.
7. CACHIN Y, JORTAY A, SANCHO H, ET AL. Preliminary results of a randomized EORTC study comparing radiotherapy and concomitant bleomycin to radiotherapy alone in epidermoid carcinomas of the oropharynx. Eur J Cancer 13:1389-1395, 1977.
8. HUSSEY DH, and ABRAMS JP. Combined therapy in advanced head and neck cancer, hydroxyurea and radiotherapy. Prog Clin Cancer 6:79-86, 1975.
9. RANDOLPH VL, VALLEJO A, SPIRO RH, ET AL. Combination therapy of advanced head and neck cancer. Induction of remissions with diamminedichloroplatinum (II), bleomycin, and radiation therapy. Cancer 41:460-467, 1978.
10. HONG WK, SHAPSHAY SM, BHUTARI R, ET AL. Induction chemotherapy in advanced squamous head and neck carcinoma with high-dose *cis*-platinum and bleomycin infusion. Cancer 44:19-25, 1979.
11. RICHMOND RC, and POWERS EL. Radiation sensitization of bacterial spores by *cis*-dichlorodiammineplatinum(II). Radiat Res 68:251-257, 1976.
12. RICHMOND RC, and SIMIC MG. Effect of radiation on *cis*-dichlorodiammineplatinum(II) and DNA in aqueous solution. Br J Cancer 37(suppl III):20-23, 1978.
13. DOUPLE EB, and RICHMOND RC. Platinum complexes as radiosensitizers of hypoxic mammalian cells. Br J Cancer 37:98-102, 1978.
14. DOUPLE EB, and RICHMOND RC. A review of platinum complex biochemistry suggests a rationale for combined platinum-radiotherapy. Int J Radiat Oncol Biol Phys 5:1335-1339, 1979.
15. WITTES RE, BRESCIA F, YOUNG CW, ET AL. Combination chemotherapy with *cis*-diamminedichloroplatinum (II) and bleomycin in tumors of the head and neck. Oncology 32:203-207, 1975.
16. ELIAS EG, CHRETIEN PB, MONNARD E, ET AL. Chemotherapy prior to local therapy in advanced squamous cell carcinoma of the head and neck: preliminary assessment of an intensive drug regimen. Cancer 43:1025-1031, 1979.
17. AL-SARRAF M, BINNS P, VAISHAMPAYAN G, ET AL. The adjuvant use of cis-platinum, Oncovin, and bleomycin (COB) prior to surgery and/or radiotherapy in untreated epidermoid cancer of the head and neck. *In* Adjuvant Therapy of Cancer III (Jones SE, and Salmon SE, eds). New York, Grune & Stratton, 1979, pp 421-428.
18. WITTES RE, CVITKOVIC E, SHAH J, ET AL. *cis*-Dichlorodiammineplatinum(II) in the treatment of epidermoid carcinoma of the head and neck. Cancer Treat Rep 61:359-366, 1977.
19. JACOBS C, BERTINO JR, GOFFINET DR, ET AL. 24-Hour infusion of cis-platinum in head and neck cancers. Cancer 42:2135-2140, 1978.
20. SAKO K, RAZOCK MS, and KALNINS I. Chemotherapy for advanced and recurrent squamous cell carcinoma of the head and neck with high and low dose cis-diamminedichloroplatinum. Am J Surg 136:529-533, 1978.
21. WITTES R, HELLER K, RANDOLPH V, ET AL. *cis*-Dichlorodiammineplatinum(II)-based chemotherapy as initial treatment of advanced head and neck cancer. Cancer Treat Rep 63:1533-1538, 1979.
22. STEPHENS R, COLTMAN C, ROSSOF A, ET AL. *cis*-Dichlorodiammineplatinum(II) in adult patients: Southwest Oncology Group studies. Cancer Treat Rep 63:1609-1610, 1979.
23. DOUPLE EB, and RICHMOND RC. Radiosensitization of hypoxic tumor cells by *cis*- and *trans*-dichlorodiammineplatinum (II). Int J Radiat Oncol Biol Phys 5:1369-1372, 1979.
24. DOUPLE EB, and RICHMOND RC. A review of interactions between

platinum coordination complexes and ionizing radiation: implications for cancer therapy. *In* Cisplatin—Current Status and New Developments (Prestayko AW, Crooke ST, and Carter SK, eds). New York, Academic Press, 1980, pp 125–147.
25. HASELOW RE, ADAMS GS, OKEN MM, ET AL. Simultaneous *cis*-platinum (DDP) and radiation therapy (RT) for locally advanced unresectable head and neck cancer. Proc ASCO 18:201, 1982.
26. DONALDSON SS. Nutritional consequences of radiotherapy. Cancer Res 37:2407–2413, 1977.

Surgical Resection and Limited Chemotherapy for Abdominal Undifferentiated Lymphomas[1]

Carol Janus, Brenda K. Edwards, Eric Sariban, and Ian T. Magrath[2,3,*]

Fourteen patients (≤ 35 years of age) with intra-abdominal undifferentiated lymphomas, without evidence of disease elsewhere, were treated by surgical resection and six cycles of combination chemotherapy which included cyclophosphamide, vincristine, doxorubicin, prednisone, and 42-hour methotrexate infusion with leucovorin rescue. One patient with very extensive retroperitoneal disease prior to surgery had rapid tumor regrowth and subsequently died. One patient has relapsed twice, but is currently alive and has been disease-free for 2 years. All other patients have remained in complete remission for 25–63 months from presentation. The major toxic effects encountered in this protocol were mucositis (which necessitated a reduction in methotrexate duration in two patients) and fever and neutropenia (which complicated chemotherapy in 15 of the total of 79 cycles delivered). There were three documented infections; all were diagnosed before chemotherapy was started, and all resolved with appropriate antibiotic treatment. The 92% actuarial survival rate in this protocol is comparable to results with more prolonged chemotherapy or a combination of prolonged chemotherapy and irradiation. Since prolonged maintenance therapy and irradiation were not utilized in our protocol, they appear to be unnecessary for patients with intra-abdominal undifferentiated lymphoma whose disease can be at least 90% resected. [Cancer Treat Rep 68:599–605, 1984]

Lymphomas are the third most common neoplasm in the US Caucasian pediatric population. Non-Hodgkin's lymphomas account for approximately 60% of these cases (1). The non-Hodgkin's lymphomas fall into three major groups: lymphoblastic, large cell (diffuse histiocytic), and undifferentiated.[4]

Most undifferentiated lymphomas in children and young adults present with intra-abdominal involvement (2). The most common sites of involvement are the terminal ileum, cecum, and appendix. Gastric and colonic involvement are rare.

Childhood undifferentiated lymphomas are characterized by a high-grade malignant growth pattern with early, wide, noncontiguous dissemination (3). Thus, treatment strategies were initially designed using chemotherapy and irradiation as primary therapies, with surgery taking an adjuvant role. However, patients with intra-abdominal disease, without evidence of disease elsewhere, have emerged as a subset of patients with undifferentiated lymphoma, for whom aggressive surgical resection should play a primary role in treatment.

The need for aggressive surgical debulking in patients with intra-abdominal lymphoma was first emphasized in 1974 in a study of African Burkitt's lymphoma (4). That study indicated a significant advantage in response to chemotherapy for patients whose tumor could be at least 90% resected. Since then, Murphy and Hustu (5) have achieved a 100% disease-free survival in eight patients with resectable intra-abdominal disease treated with an intensive induction regimen, irradiation, and 2 years of maintenance therapy.

The purpose of this paper is to report a group of patients with intra-abdominal undifferentiated lymphomas treated with surgical resection and six cycles of combination chemotherapy given at 3–4-week intervals. Our therapy is unique, in that irradiation was not given and chemotherapy was intense but of limited duration.

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[4]Magrath IT, Janus C, Edwards B, et al. An effective therapy for both undifferentiated lymphomas (including Burkitt's) and lymphoblastic lymphomas in children and young adults. *Blood*, in press.

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PATIENTS AND METHODS

Between June 1977 and February 1981, 59 consecutive, previously untreated patients were entered in the National Institutes of Health (NIH) Pediatric Oncology Branch Non-Hodgkin's Lymphoma Protocol 77-04. A histologic diagnosis of non-Hodgkin's lymphoma and age < 35 years were the criteria for entry into the protocol. The protocol was reviewed and approved by the Investigational Review Board, with informed consent being obtained from the parents and, when appropriate, from the patients.

All patients with resected disease had their initial workup at the National Cancer Institute (NCI) completed within 3–4 days, prior to starting treatment. All other patients were admitted as soon as possible and had their workup completed within 24–48 hours. All patients had a cbc with differential, rbc indices, and platelet count. Serum chemistries included electrolytes, uric acid, LDH, and liver and renal function studies. Appropriate cultures were taken when fever was present. Roentgenographic studies included chest x-ray (posteroanterior and lateral), iv pyelogram, and computerized transaxial tomography of the abdomen and cranium. Ultrasound of the kidneys, abdomen, and pelvis was performed on all patients. Nuclear medicine studies included gallium-67 and technetium bone scans. Bilateral bone marrow biopsies and aspirates and examination of the cerebrospinal fluid were routinely performed on each patient. Pleural or ascitic fluid, if present, was aspirated and examined for malignant cells. Lymphangiography was not done.

Upon completion of the initial workup, all patients were prospectively assigned a stage according to the following classification originally devised for African Burkitt's lymphoma: A—single extra-abdominal tumor; B—multiple extra-abdominal tumors; C—intra-abdominal tumor; D—intra-abdominal tumor and extra-abdominal tumors; and AR—patients in whom > 90% of intra-abdominal tumor is resected. All 14 patients reported in this study were classified as stage AR prior to initiating treatment. Twelve of these 14 patients had surgery at their local hospital prior to referral to NIH. Assignment of the patient to stage AR was based on the judgment of the surgeon as to whether at least 90% of the tumor had been resected and also on the initial workup which, by definition, did not reveal evidence of disease in patients referred postoperatively.

All patients were also assigned a histologic diagnosis prior to the start of therapy. All diagnoses were made by the NCI pathologists on available biopsy material.

Treatment consisted of surgery for patients with potentially resectable disease and chemotherapy for all patients. Radiation therapy was not a part of the primary treatment. The chemotherapy consisted of cyclophosphamide (1200 mg/m^2 iv), doxorubicin (40 mg/m^2 iv), vincristine (1.4 mg/m^2 iv; maximum total dose, 2.0 mg), and prednisolone or prednisone (40 mg/m^2 daily for five doses iv or orally) followed by a 42-hour methotrexate infusion (2.7 g/m^2, total) starting on Day 10, with subsequent leucovorin rescue (fig 1). The first cycle consisted of cyclophosphamide only, on Day 1, and the 42-hour methotrexate infusion starting on Day 10. Leucovorin rescue consisted of a dose of 48 mg/m^2 iv at the end of the infusion and 12 mg/m^2 every 6 hours, until the methotrexate level was $< 5 \times 10^{-8}$ M. Cycles were started as soon as marrow recovery occurred (arbitrarily defined as 1500 granulocytes/mm^3).

An apparent high frequency of isolated CNS relapses occurred among the first 13 patients entered into the protocol. This resulted in modification of the protocol to include an intensive intrathecal regimen consisting of cytarabine and methotrexate (fig 1). Although none of the five stage AR patients in the initial 13 had relapsed in the CNS, a decision was made to continue to treat all patients uniformly with CNS prophylaxis. The small number of patients entered in the protocol (19 patients) and the small number of CNS relapses (four) precluded making significant observations as to which groups of patients would most benefit from CNS prophylaxis.

Patients with stages A, B, or AR disease were given six cycles of therapy. Patients with more extensive disease (stages C and D) were given 15 cycles of chemotherapy.

All patients were routinely re-staged at 2–3-month intervals. Re-staging measurements included cbcs, serum chemistries, computerized transaxial tomography and ultrasound of the abdomen, gallium-67 and technetium bone scans, bilateral bone marrow biopsies and aspirates, and examination of the cerebrospinal fluid. A "second-look" laparotomy was not a standard part of follow-up.

Once therapy was discontinued patients were followed monthly for three visits, every other month for two visits, every third month for two visits, and then every 6 months over a 3-year period from the end of treatment. Patients were then seen yearly for follow-up. After remaining disease-free for 1 year after completion of therapy, further studies were performed only if indicated.

RESULTS

Forty of the 59 patients admitted had undifferentiated intra-abdominal lymphoma. Twenty-six of these patients had either unresectable disease (stage C) or additional disease at distant sites (stage D). Fourteen patients had resected intra-abdominal disease (stage AR) and are the subject of this report.

Table 1 shows the age, sex, histology, disease site, and remission duration for the 14 patients. The ages ranged from 3 to 35 years, with a median age of 14 years. The male:female ratio of 6:1 was higher than the reported ratio of 3:1 (3). The predominant histologic type was Burkitt's lymphoma (11 patients). The most common disease site was the ileal or ileocecal region (12 patients). The

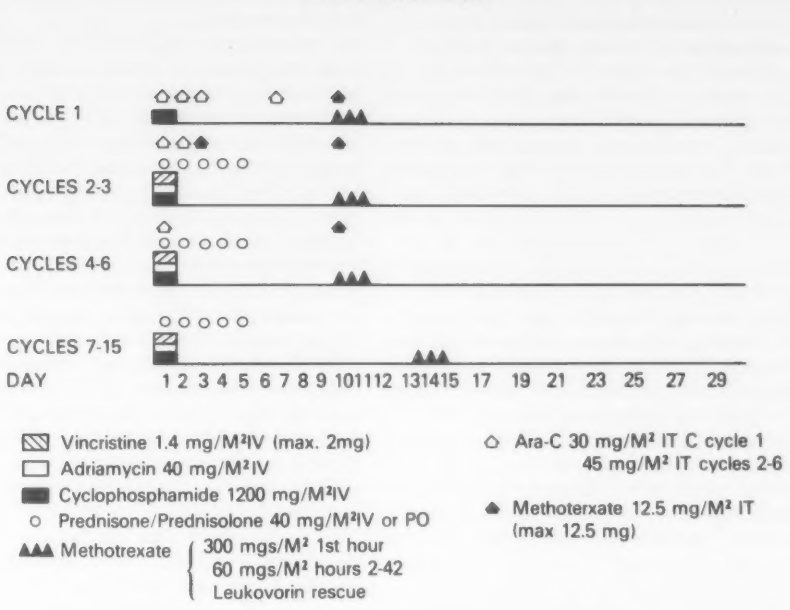
PROTOCOL 77-04
CYCLE 1
CYCLES 2-3
CYCLES 4-6
CYCLES 7-15
DAY
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 17 19 21 23 25 27 29
Vincristine 1.4 mg/M²IV (max. 2mg)
Adriamycin 40 mg/M²IV
Cyclophosphamide 1200 mg/M²IV
Prednisone/Prednisolone 40 mg/M²IV or PO
Methotrexate
300 mgs/M² 1st hour
60 mgs/M² hours 2-42
Leukovorin rescue
Ara-C 30 mg/M² IT C cycle 1
45 mg/M² IT cycles 2-6
Methoterxate 12.5 mg/M² IT
(max 12.5 mg)
Cycles commence as soon as granulocytes over 1500/cu. mm (or day 28
cycles 7-15)

FIGURE 1.—Treatment scheme.

other primary sites were kidney, ovaries, and right groin, with tumor extending into the inguinal triangle and retroperitoneally. The smallest tumor was 2 × 2 cm and the largest was 20 × 20 × 25 cm.

Eight patients presented with an acute abdomen, with the etiology either unspecified or thought to be appendicitis. Two patients were treated with prednisone for ileitis, one for 1 month and the other for 8 months. The patient treated for 8 months eventually developed a perforation of the bowel and a pelvic abscess. Two patients (18 and 4 years old) presented with large, palpable masses and were considered to have tumor (unspecified and neuroblastoma, respectively). The preoperative diagnosis in the patient with renal disease was perirenal abscess or hematoma.

The most common presenting symptom was pain, a chief complaint of all 14 patients. Other common symptoms were weight loss (six patients), vomiting, secondary to obstruction (five patients), and fever (four patients). Two patients with involvement of the terminal ileum experienced melena. The patient with kidney involvement had hematuria and the patient with ovarian involvement reported "hot flashes." The duration of symptoms ranged from 24 hours to 9 months.

Positive findings on physical examination partially depended on whether obstruction or perforation had occurred. The five patients with obstruction all appeared acutely ill on examination. Two of these patients had intussusception. Three patients had perforations of the bowel and developed marked tenderness, rebound, and a tense abdomen. Six patients had a readily palpable mass. Hepatosplenomegaly was not present in any of these patients.

Twelve of the 14 stage AR patients had resection at the referring institution. One of these patients had complete resection of multiple ileocecal masses but experienced regrowth of tumor in the ovaries and peritoneum in the 8-week interval before presentation to NIH. She underwent bilateral salpingo-oophorectomy at NIH prior to starting chemotherapy. Tumor was scraped off the uterus, but a hysterectomy was not done.

Of the other two patients who underwent resection at NIH, one had had prior orchiectomy for diagnosis and was operated on at NIH on an emergency basis because of perforation. The other patient had disease of the right groin infiltrating the skin, filling the inguinal triangle and extending retroperitoneally. This had been biopsied but was thought to be unresectable by the surgeons at the

referring institution.

Thirteen of the 14 patients are alive. Twelve of the patients never relapsed, are off therapy, and are free of disease between 25 and 63 months after the start of therapy. One of the survivors has relapsed twice, the first time after four cycles of therapy. This relapse was diagnosed on the basis of a small nodule of tumor cells seen in one bone marrow clot section only. Bilateral bone marrow biopsies and marrow aspirate smears were free of tumor cells. The patient was treated with high-dose cyclophosphamide and cytarabine and remained free of disease for 39 months after relapse (43 months after diagnosis). He subsequently developed crampy abdominal pain, diarrhea, a 10-pound weight loss, and a right lower quadrant mass. At surgery, he was found to have lymphomatous involvement of part of the remaining ileum (his site of involvement at presentation) and cecum, which were resected. He is currently free of disease after 2 years without treatment, following six additional cycles of chemotherapy. Routine re-staging of the other 12 survivors has failed to reveal evidence of disease recurrence.

The one partial responder had had a thigh mass which extended along the iliopsoas muscle and the iliac vessels and involved the right inguinal lymph nodes. He continued to have abnormal uptake in the right inguinal area on his postoperative gallium scan. This progressively worsened and he had obvious regrowth of tumor at the presenting site between his second and third cycles of therapy. He was treated with high-dose cyclophosphamide and cytarabine but developed *Escherichia coli* sepsis and hemorrhage and died 4 months after diagnosis. There was no residual tumor at autopsy.

It should be reiterated that this patient had a very large (20 × 20 × 25 cm) tumor which infiltrated through skin, into muscle, and involved the femoral nerve, artery and vein, the inguinal ligament, right inguinal lymph nodes, obturator nodes, and the saphenous vein. Although a massive amount of gross tumor was removed, residual tumor was almost certainly present in multiple structures (muscle, retroperitoneal tissue) not resected. This patient was included in this study because his disease predominantly involved the retroperitoneum and because the surgeon believed that at least 90% of the tumor had been resected. He was classified as stage AR prior to chemotherapy. However, the determination of whether 90% of the tumor had been removed was, in his case, a difficult one. He is probably in a different category from the other patients and, in retrospect, might better have been classified as stage D.

The toxic effects and complications associated with this treatment protocol were both predictable and manageable. All patients experienced alopecia and some degree of nausea and vomiting. The nadir myelosuppression usually occurred either during or after the methotrexate

TABLE 1.—Patient characteristics

Age (yrs)	Sex	Disease site(s)	Histology	Remission duration (mos)
18	M	Right thigh, filling inguinal triangle, extending retroperitoneally	Burkitt's	0
8	M	Ileal mass, sigmoid	Undifferentiated, unspecified	4
10	M	Ileocecal valve	Burkitt's	25+
14	M	Terminal ileum	Burkitt's	26+
8	M	Ileocecal valve	Burkitt's	30+
7	M	Terminal ileum	Burkitt's	38+
16	M	Proximal ileum, adherent to bladder, involving rectosigmoid	Burkitt's	40+
16	M	Mid-small bowel	Burkitt's	40+
16	M	Omentum, visceral peritoneum, small bowel and mesentery, most of cecum (studded peritoneum not resected)	Burkitt's	41+
14	F	Terminal ileum, cecum, adjacent nodes	Undifferentiated, non-Burkitt's	45+
3	M	Lower ileum, ileocecal valve	Burkitt's	46+
10	M	Ileum, involving dome of bladder, mesentery	Burkitt's	56+
35	M	Kidney	Burkitt's	61+
34	F	Ileocecal valve, cecum, multiple small bowel mesenteric nodes; regrowth: ovaries, peritoneum	Burkitt's	63+

infusion and was sometimes associated with mucositis. The median nadir wbc count was 1300/mm^3 (range, 300–2400).

Severe neutropenia and mucositis resulted in omission of the methotrexate infusion during the last cycle for one patient and in shortening of the infusion to 36 hours for two patients, both for the last four cycles. One of these patients also had a 25% reduction of cyclophosphamide and doxorubicin during the last cycle. Two patients had a 25% reduction in cyclophosphamide and doxorubicin during the last four cycles because of severe neutropenia and mucositis. In additon, one patient had a 25% reduction in doxorubicin during the last two cycles because of severe mucositis. Eight patients had no dosage modifications.

There were 15 episodes (in a total of 79 cycles) of fever associated with neutropenia (polymorphonuclear neutrophil counts < 500/mm^3) that resulted in administration of parenteral antibiotics. Six patients never had a fever during treatment. There were three episodes of documented infection, all of which began before chemotherapy was administered. Two patients developed peritonitis, one due to *Clostridia* infection and the other due to multiple organisms including Group D streptococcus, *Klebsiella*, and *E. coli*. The third infection was a sinus infection. All resolved completely after antibiotic administration. One patient developed intestinal obstruction 4 months after surgery. Laparotomy revealed multiple adhesions, with no evidence of recurrent disease. Postoperatively, the patient developed a pelvic abscess which required drainage. His subsequent clinical course has been uneventful and he has remained well for 36 months.

No doxorubicin toxicity was encountered in this protocol.

DISCUSSION

The results of the protocol described re-emphasize the need for an aggressive surgical approach in young patients with intra-abdominal undifferentiated lymphomas. In our study, each patient with intra-abdominal disease was evaluated for feasibility of surgical resection. The tumor burden varied markedly from patient to patient, but extensive disease was not a contraindication to surgery, unless the surgery was technically not possible. In addition, in one case, rapid regrowth of a large tumor involving multiple structures (both ovaries and perito-

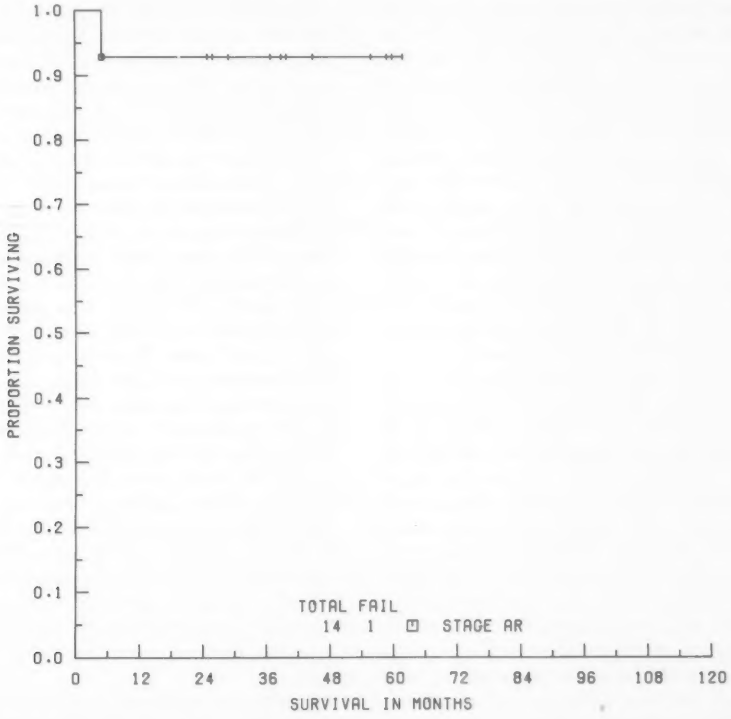

FIGURE 2.—Kaplan-Meier plot of survival for 14 patients with surgically resected undifferentiated abdominal lymphoma.

neum) was not considered a contraindication to repeat resection of disease prior to initiating chemotherapy.

Similar results to ours have been obtained in other centers using more intensive or more prolonged therapy. In a recent prospective trial carried out at St. Jude Hospital, an intensive induction regimen, whole-abdominal irradiation, and 2 years of maintenance chemotherapy were utilized (5). A 100% 2-year, disease-free survival rate in eight patients with resected abdominal disease was achieved. Since patients with resected abdominal disease had a low relapse hazard, the authors questioned whether they would do as well with less intense therapy. They also discussed the problems associated with using large-volume, conventional-dose radiotherapy in combination with a multiple-drug regimen. In a prospective study carried out at Memorial Sloan-Kettering Cancer Center, treatment consisted of surgical resection and combination chemotherapy given on an induction, consolidation, and maintenance schedule over 2–3 years (6). In addition, three patients with residual disease were given radiation therapy. The authors reported a 72% overall survival rate, which included patients with unresectable disease. Since these patients are a poorer prognostic group, one can probably assume that the survival rate for patients with resected disease was > 72%.

The 92% disease-free survival (fig 2) obtained with our protocol compares well with these other prospective clinical trials (5,6) but is unique in two aspects. First, irradiation was not a part of treatment, which obviates both the short-term and long-term toxic effects encountered when irradiation and combination chemotherapy are administered together. Second, patients were treated for six cycles over approximately 5–6 months, unlike other non-Hodgkin's lymphoma protocols for young people which are patterned after leukemia protocols that extend treatment over 2–3 years. The short-term benefits of limited-duration chemotherapy include fewer days at risk for neutropenia-associated infections and sepsis and fewer days spent in the hospital. The psychosocial benefits of fewer days spent in the hospital are considerable.

The long-term complications of therapy are particularly important in this group of patients with a potential for cure. Such complications include long-term effects on the reproductive, cardiovascular, immune, and nervous systems. The potential oncogenic effect of the therapy is also of concern. Whatever the mechanisms of long-term toxic effects and oncogenesis, the cumulative drug and/or radiation dose and the duration of therapy are likely to be important factors. Thus, it is important to determine whether even less chemotherapy would result in as good survival with less toxicity. Patients in the Pediatric Branch's two previous non-Hodgkin's lymphoma protocols (7–9) were treated with only three cycles of combination chemotherapy followed by abdominal irradiation. Unfortunately, the number of surgically resected patients in these protocols is too small to make statistically significant comparisons, although two of five patients died. Attempts to reduce therapy further should, in any event, be made with considerable caution, and only changes likely to make a significant improvement in morbidity should be considered. For example, only 200 mg/m^2 of doxorubicin is given in protocol 77-04, and although the contribution of this drug cannot be assessed, the very low risk of cardiac toxicity would not make its exclusion in future trials a high priority.

The extent to which the intensive intrathecal CNS prophylaxis, which ten of these 14 patients received, may have contributed to survival is unknown. However, Nelson et al (10), in a retrospective study of 40 patients with resected intra-abdominal non-Hodgkin's lymphoma, found that CNS involvement occurred only during the terminal phase of the disease and was never the first site of occurrence. The 90% survival rate reported in patients treated with surgical resection, abdominal irradiation, and chemotherapy, without CNS prophylaxis, also suggests that CNS prophylaxis may not be necessary for patients with resected disease. Although no significant short- or long-term morbidity (other than headache) has been observed, omission of the intensive CNS prophylaxis therapy would have contributed to the patients' comfort and to the ease of administration of the protocol. Further, rare cases of chemical arachnoiditis have been observed in other patients treated with protocol 77-04. This, however, must be weighed against the presumed small risk of CNS relapse, and an appropriate course of action would be to reduce intrathecal therapy to perhaps five or six injections of cytarabine and methotrexate.

One of the most difficult issues to address is that of defining which patients would benefit from an attempt at surgical resection. We have based our determination on an extensive but rapid workup and, in general, have not attempted to resect large, fixed masses arising from the retroperitoneum. This was done in only one case and resulted in ultimate failure. It is probable that even the most aggressive procedure may fail to remove sufficient tumor in such cases. However, it is sometimes not possible to determine whether tumor is resectable until surgery is actually attempted, and, for this reason, we would advocate exploratory surgery in all cases of tumor confined to the abdomen excepting those where total resection is clearly impossible because of the anatomic position of the tumor. Clinical examination and computerized axial tomography usually provide sufficient information for this determination to be made. The general surgeon faced with an undiagnosed tumor laparotomy would be advised to adopt an aggressive approach with a view to resecting all tumor, if possible, particularly if limited to the gastrointestinal tract, mesentery, or an expendable organ.

In summary, complete resection and six cycles of combination chemotherapy resulted in a 92% disease-free survival in 14 patients with intra-abdominal undifferen-

tiated lymphoma. Since prolonged maintenance and irradiation were not utilized, they appear to be unnecessary for patients whose disease can be at least 90% resected.

REFERENCES

1. MURPHY SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphomas: dissimilarities from lymphomas in adults. Semin Oncol 7:332–339, 1980.
2. MAGRATH IT. Malignant lymphomas. *In* Cancer in the Young (Levine AS, ed). New York, Masson Publishing Inc, 1982, p 509.
3. MURPHY SB. Hodgkin's disease and the non-Hodgkin's lymphomas in childhood. Semin Oncol 1:17–26, 1974.
4. MAGRATH IT, LWANGA S, CARSWELL W, ET AL. Surgical reduction of tumor bulk in management of abdominal Burkitt's lymphoma. Br Med J 2:308–312, 1974.
5. MURPHY SB, and HUSTU HO. A randomized trial of combined modality therapy of childhood non-Hodgkin's lymphoma. Cancer 45:630–637, 1980.
6. WOLLNER N, WACHET AE, EXELBY PR, ET AL. Improved prognosis in children with intra-abdominal non-Hodgkin's lymphoma following LSA_2L_2 protocol chemotherapy. Cancer 45:3034–3039, 1980.
7. ZIEGLER JL. Treatment results of 54 American patients with Burkitt's lymphoma are similar to the African experience. N Engl J Med 297:75–80, 1977.
8. ZIEGLER JL, MAGRATH IT, DEISSEROTH AB, ET AL. Combined modality treatment of Burkitt's lymphoma. Cancer Treat Rep 62:2031–2034, 1978.
9. ZIEGLER JL, DEVITA VT, GRAW RG, ET AL. Combined modality treatment of American Burkitt's lymphoma. Cancer 38:2225–2231, 1976.
10. NELSON DF, CASSADY JR, TRAGGIS D, ET AL. The role of radiation therapy in localized resectable intestinal non-Hodgkin's lymphoma in children. Cancer 39:89–97, 1977.

Phase I Trial of *N*-Methylformamide[1]

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***N*-methylformamide was administered iv and later orally to 19 patients in a phase I study, with a starting dose of 300 mg/m²/day × 5. The cycles were planned to be repeated every 2 weeks, and doses were escalated in four steps to 1200 mg/m²/day × 5. The principal toxic effect of the drug was nausea and vomiting, but this was not severe enough to interfere with oral medication. Parallel bioavailability studies confirmed excellent absorption of the drug. Biochemical disturbances included reversible elevation in transaminases not related to dose, peripheral neuropathy in one patient, and drug interaction with alcohol in another. The dose-limiting toxic effect was reversible hyperbilirubinemia. One patient became jaundiced. Among 15 evaluable patients, there were two partial responses (prostate and cervix carcinoma) and two minimal responses (ovarian carcinoma and hypernephroma). The recommended dose for the phase II study is 800 mg/m²/day × 5 orally, repeated every 2 or 3 weeks.**
[Cancer Treat Rep 68:607–610, 1984]

N-methylformamide (NMF) was first recognized as an antitumor agent in 1953. It was taken into clinical trial with five patients and then discarded because of the occurrence of reversible hepatotoxicity (1). Interest in the drug has been reawakened by a structure-activity study of various *N*-methyl substances, of which NMF appeared to be one of the most potent compounds. It was tested in the National Cancer Institute human tumor xenograft panel and showed antitumor activity in the MX 1 mammary tumor, CX 1 colon tumor, and LX1 lung xenografts. The drug has only moderate activity against L1210 and P388 leukemias (the common prescreening models), and is effective against murine sarcoma 180, M5076 ovarian sarcoma, TLX5 lymphoma, AKR leukemia, and Ehrlich ascites carcinoma (2,3). While *N*-ethylformamide has been shown to be equitoxic to the *N*-methyl compound in the TLX5 lymphoma in vitro, neither *N*-ethylformamide nor formamide (a metabolite of NMF in mice) showed in vivo activity (4).

On the basis of the human tumor xenograft data, the likely absence of myelotoxicity (5), and the paucity of data on the drug's effect in humans, it was decided to activate a formal phase I study. A 5-day schedule repeated every 2–3 weeks was chosen, because it resembled the best dose schedule for treatment of the mouse ovarian sarcoma (3).

MATERIALS AND METHODS

NMF was formulated in ampules for iv use; contents of the ampules were mixed with orange juice for oral administration in the first ten patients. The first three patients were treated with a single dose of NMF at 300, 600, and 1200 mg/m² weekly, administered orally on Week 1 and iv on Week 2 to establish preliminary pharmacokinetics (reported elsewhere). The next 19 patients were treated with doses starting at 300 mg/m²/day × 5 (10% of the LD10 in mice) followed by escalation in gradual increments (table 1).

The first seven patients received the drug orally and then 3 weeks later iv (at the same dose) to provide further pharmacokinetic profiles and to determine the bioavailability. The results of this study suggested that absorption of the drug was excellent after oral ingestion, so the remaining 12 patients were treated exclusively with oral NMF. Patients participating in the study were considered to have failed conventional therapy; they had a life expectancy > 6 weeks; had adequate bone marrow, renal, and hepatic reserve; and gave written informed consent. The following biochemical pretreatment values were required: Hgb > 7.0 mmols/L, leukocytes > 3000/mm³, platelets > 100,000/mm³, creatinine < 80 μmols/L, SGOT/SGPT < 30 IU, and bilirubin < 2 μmols/L.

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TABLE 1.—Dose escalation scheme*

Dose (mg/m^2/day × 5)	No. of patients	No. of cycles
300	4	15
600	5	10
800	3	5
1000	7	18
1200	1	2
Total	20	50

*There was 1 wk between each patient and 2 wks between each escalation. 1 patient was treated with 2 doses.

In all cases, an interval of at least 3 weeks after completion of previous chemotherapy or radiotherapy (6 weeks for patients treated with mitomycin or nitrosoureas) was required before NMF treatment was started. The patient characteristics are described in table 2, including pretreatment performance status. The patients were treated in a clinical research unit; only towards the end of the study were three patients treated on an outpatient basis. Apart from the routine clinical history and examination, hematological and biochemical profiles were evaluated weekly, at which time patients were asked to respond to a questionnaire to elucidate toxicity. Toxic effects were subsequently graded according to World Health Organization (WHO) criteria.

RESULTS

Nineteen patients, aged 19–72 (median, 52), were admitted to the trial, but one has not been further evaluated because of rapid deterioration of health, intestinal obstruction associated with tumor progression, and interruption of the first course of NMF on Day 3. There were 12 females and seven males in the study. The number of patients at each dosage level and the number of cycles are shown in table 1. One patient was treated at two dose levels. Approximately one-third of the cycles were associated with toxicity. The most frequent toxicity pattern was anorexia and nausea, with occasional vomiting (table 3). In one patient, this was severe enough to merit active treatment. Though a general feeling of tiredness and sleepiness was common, the drug was surprisingly well tolerated. One patient developed a peripheral sensorimotor neuropathy predominantly affecting the lower limbs, which was severe enough to cause alteration in gait. This neuropathy was totally reversed within 4 weeks of stopping the drug. It is possible that the neuropathy was not a direct effect of the drug, because 2 weeks before the administration of NMF, the patient had undergone alcohol embolization of a massive left kidney tumor. Whereas this was not considered at the time to constitute significant antitumor therapy and, therefore, ground for exclusion from the trial (the patient had measurable lung metastases), a repeat computerized tomographic (CT) scan of the kidney 2 weeks after treatment with NMF and at the onset of the peripheral neuropathy showed complete infarction of the kidney. The patient was subsequently re-treated with NMF at his own request and, after 5 months of treatment, has had no recurrence of his neuropathy. Workers exposed to *N*, *N*-diethylformamide, the methyl homolog of NMF, have complained of disulfiram-like reactions after drinking alcohol (6), so it was of interest that one patient, while receiving NMF, reported the onset of severe facial flushing shortly after drinking a glass of wine. Interaction with alcohol may prove to be a problem. Another patient experienced repeated alteration in auditory acuity, coincident with the administration of NMF in seven of eight cycles. He described an awareness that all noises, no matter how close, sounded as if they were coming from a far distance. Repeated audiograms showed no abnormality.

The principal toxicity noted was biochemical; whereas no indications of renal or bone marrow toxicity were seen, reversible elevation in transaminases was noted from the first dosage level (300 mg/m^2 × 5). Rises in SGOT and SGPT were seen in one-third of all patients, commonly occurring around Day 5 and returning to normal by Day 10. There was no dose-dependent pattern.

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Age range (yrs): 19–72	
Diagnosis	
Ovarian carcinoma	5
Colorectal carcinoma	4
Hypernephroma	3
Sarcoma	4
Prostate carcinoma	1
Cervix carcinoma	2
Previous treatment	
Single-agent chemotherapy	8
Combination chemotherapy	7
Radiotherapy + chemotherapy	2
No previous therapy	2
Performance status (WHO)	
1	10
2	7
3	2
Male/female ratio	7/12
Total	19

TABLE 3.—Side effects after 50 cycles

Side effect	WHO grade	No. of cycles
Nausea	⩽ 2	17
Nausea/vomiting	3	3
Anorexia	⩽ 2	11
Sleepiness	2	14
Phlebitis		2
Alcohol flush		1
Hepatomegaly		6
Peripheral neuropathy		1

What was more serious and was considered to be the dose-limiting toxic effect of the study was the onset of hyperbilirubinemia, associated with increasing alkaline phosphatase levels, which was sporadically observed at the lower dosage levels but was seen in nine of 18 cycles at 1000 mg/m^2 daily (table 4). This obstructive pattern of liver function tests was not always accompanied by elevated transaminases, was always reversible, and led to symptoms and jaundice in only one patient. She became clinically jaundiced on two occasions associated with the drug and on each occasion, marked hepatomegaly was found upon clinical examination. This hepatomegaly receded in the intervals between therapy cycles and increased the difficulty in evaluation of the tumor, because the liver was the principal site of measurable disease. The patient suffered from colon carcinoma metastastic to lung and liver and insisted on continuing with therapy despite the jaundice, because her hepatic pain improved after each course of therapy. It is of considerable interest that, in this patient and in three others treated at the highest dose, levels of gamma glutamyl transferase (γGT) fell as a result of NMF therapy (11 of 18 cycles).

Two liver biopsy specimens were obtained from the jaundiced patient and one was obtained from a patient who showed no visible impairment of liver function until after his fifth course of NMF, when bilirubin temporarily rose in association with increasing alkaline phosphatase levels. This patient also had measurable liver metastases on CT scan associated with marked liver pain; the primary tumor was a hypernephroma. He too reported marked and prompt relief of pain from the first cycle of NMF; in fact, he returned to semiactive work for 2 months after the beginning of the phase I study. Throughout 3½ months of observation, the liver metastases were shown on echo to regress slightly and to become necrotic, whereas the lung metastases, which were also easily measurable, showed no change.

The liver biopsy specimens were reported to consist of swollen hepatocytes with some pyknosis and a nonspecific periportal inflammatory infiltrate. No evidence of cirrhosis was seen.

One patient who had cytologically positive, measurable lymph node metastases from prostate cancer achieved partial remission, accompanied by an improvement in bone pain and a fall in acid phosphatase from 40 times the normal value to twice the normal value. However, this patient had coincident brain metastases which were subjected to palliative radiation treatment without success. Three months later, the patient died of progressive brain metastases without further evidence of progression of his measurable peripheral metastases.

A second partial remission was achieved in a 55-year-old patient (WHO status 1) with carcinoma of the uterine cervix, who had measurable metastases in lung, pleura, and liver. She had recently progressed after an initial excellent partial response of 6 months' duration following mitomycin, vincristine, bleomycin, and cisplatin, marked by surprisingly little toxicity. After the first course of NMF, there was obvious improvement in her chest x-ray, but this was not confirmed as a partial remission until after a second course, at which time a liver CT scan was repeated and confirmed response also in that organ. The response lasted 5 months. This patient developed painless hepatomegaly on Day 4 or 5 of every treatment cycle, which disappeared around Day 10. However, she had no alteration of any liver function tests, and the only side effects were nausea and anorexia coinciding with her enlarged liver.

Two minor responses were noted in ovarian cancer and hypernephroma. The patient with ovarian cancer had previously had disease progression during a cisplatin combination regimen and, thereafter, during therapy with a platinum analog, TNO 6, in a phase I trial. The measurable lesion was a cytologically positive groin lymph node, which shrank by 40% of its volume after one course of NMF and then remained unchanged throughout three further courses. A liver scan demonstrated stable metastases. Lastly, a patient with hypernephroma had a 20% regression of lung and liver metastases, which was sustained for seven courses of therapy. Response data are summarized in table 5.

TABLE 4.—Liver function test abnormalities

Dose mg/m^2/day	Route	Total No. of cycles	No. of cycles causing elevation to greater than twice the normal values			
			SGOT	Alkaline phosphatase	Bilirubin	γGT
300	Iv	15	5	1	1	1
600	Iv	3	0	0	0	0
600	Oral	7	2	1	1	1
800	Oral	5	0	1	0	0
1000	Oral	18	5	8	9	2*
1200	Oral	2	0	2	2	0
Total		50	12	13	13	4

* γGT was decreased in 11 cycles.

TABLE 5.—Patient responses

Response	No. of patients
Partial response	2
Minimal response	2
Stable	2
Progression	9
Not evaluable	4

DISCUSSION

The demonstration of clinical antitumor activity, though modest, is important for two reasons: it supports the prediction of the National Cancer Institute human xenograft panel which, in the case of NMF, contradicted the usual prescreening L1210 and P388 models. Second, and perhaps not unrelated to the inactivity in the murine leukemias, NMF joins the diminutive ranks of nonmyelosuppressive agents, which are particularly important for inclusion in combinations of cytotoxic drugs. The toxicity of the drug was acceptable, particularly in light of the fact that more than one-half of the patients in the trial had previously undergone treatment with another phase I agent. The rise in transaminases did not appear to be dose-related, nor was it dependent on the mode of administration of the drug. It was always rapidly reversed and was not associated with hepatomegaly. Hepatomegaly was indeed observed in several patients who exhibited normal liver function tests throughout the entire course of treatment. More serious, however, was the occurrence of an obstructive pattern of liver function disorder, the mechanism of which has yet to be elucidated. Liver biopsy carried out in two patients was unhelpful, showing nonspecific inflammatory changes. Histology of the liver and other organs was obtained at autopsy in several patients and was normal, apart from the presence of tumor, which was generally the cause of death. Three patients have survived a year after the phase I trial. All three have gone on to participate in another phase I trial of another drug and, at present, they show no evidence of chronic liver disease. Peripheral neuropathy may prove to be a side effect of this drug. As discussed above, there is a clear-cut instance of a drug interaction between NMF and alcohol in one patient, resembling a disulfiram effect. Further trials of this drug as a single agent and in combinations would appear appropriate, and at least three further phase I studies are under way, testing the drug in different schedules, orally and iv.

REFERENCES

1. MEYERS WPL, KARNOFSKY D, and BURCHENAL JH. The hepatotoxic action of *N*-methylformamide in man. Cancer 9:949-955, 1956.
2. CLARKE CA, PHILLIPS RS, STERNBERG SS, ET AL. Effects of *N*-methylformamide and related compounds in sarcoma 180. Proc Soc Exp Biol Med 84:203-207, 1953.
3. GESCHER A, GIBSON NW, HICKMAN JA, ET AL. *N*-methylformamide: antitumour activity and metabolism in mice. Br J Cancer 45:843-850, 1982.
4. BRINDLEY C, GESCHER A, HARPUR ES, ET AL. Studies of the pharmacology of *N*-methylformamide in mice. Cancer Treat Rep 66: 1957-1965, 1982.
5. NEWMAN KN, MEEKS RG, and FRICK S. Phase I Report of Task 1 Preclinical Intravenous Toxicity Study of *N*-Methylformamide (NSC3051) in CDF_1 Mice and Beagle Dogs. Birmingham, AL, Southern Research Institute, 1981.
6. LYLE WH, SPENCE TWM, MCKINNELY WM, ET AL. Dimethylformamide and alcohol intolerance. Br J Ind Med 36:63-66, 1979.

Predictive Tests for the Response to Cancer Chemotherapy: Limitations Related to the Prediction of Rare Events[1,2]

Marcel Rozencweig[3,*] and Maurice Staquet[4,5]

The reliability of a predictive test may be characterized by its sensitivity and specificity. The probability that a predicted event will actually occur depends on these indexes as well as the frequency of the predicted event. If such a test is used to predict for the therapeutic effect of single-agent chemotherapy in cancer patients, its predictive value is markedly affected by a generally low therapeutic efficacy in an unselected population. [Cancer Treat Rep 68:611–613, 1984]

Prospective studies indicate that drug testing in a cloning assay might be successfully used for defining optimal chemotherapy regimens in individual cancer patients (1,2). Striking in vitro-in vivo correlations in these studies have raised considerable interest in chemosensitivity tests. Our purpose is to underline limitations inherent to the prediction of clinical drug effect in cancer patients, as previously discussed elsewhere (3,4). This analysis is illustrated with arbitrarily selected figures, assuming that the test results and the clinical effect of the treatment may be expressed dichotomously as "positive" or "negative" for drug efficacy or resistance, respectively.

Characteristics of a Predictive Test

The reliability of a predictive test may be defined by its *sensitivity* and *specificity* (5,6). *Sensitivity* is the ability of the test to identify patients whose disease responds clinically to chemotherapy, whereas *specificity* is the ability of the test to identify patients whose disease does not respond clinically. These indexes characterize the test with one regimen and one tumor type for fixed criteria of experimental and clinical response. They are determined during preliminary evaluation of the test and are calculated as follows:

$$\textit{Sensitivity} = \frac{\text{Patients with clinically responsive disease and positive test}}{\text{All patients with clinically responsive disease}} \times 100$$

$$\textit{Specificity} = \frac{\text{Patients with clinically refractory disease and negative test}}{\text{All patients with clinically refractory disease}} \times 100.$$

If the test is then used to predict clinical results of chemotherapy, it is essential to know its *predictive value*. The *predictive value* of a positive test (PV pos) gives the probability that a patient with a positive test will achieve clinical response, whereas the *predictive value* of a negative test (PV neg) gives the probability that a patient with a negative test will fail to achieve clinical response. These indexes are calculated as follows:

$$\text{PV pos} = \frac{\text{Patients with clinically responsive disease and positive test}}{\text{All patients with positive test}} \times 100$$

$$\text{PV neg} = \frac{\text{Patients with clinically refractory disease and negative test}}{\text{All patients with negative test}} \times 100.$$

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The *predictive value* cannot be determined directly from the *sensitivity* and *specificity*, since it is also related to the probability of response in the entire population under study (5,6). This probability may be estimated from past experience providing a historical response rate in this population. A lower expected response rate results in a reduced *predictive value* of a positive test and an increased *predictive value* of a negative test, despite unchanged *sensitivity* and *specificity*, as expressed mathematically by the following equations (5):

$$PV\ pos = \frac{p \times sensitivity}{p \times sensitivity + (1-p)(1-specificity)} \times 100$$

$$PV\ neg = \frac{(1-p) \times specificity}{(1-p) \times specificity + p(1-sensitivity)} \times 100.$$

In these equations, p represents the probability of response for a patient in the population under study. It is readily apparent that, when p tends to zero, the *predictive value* of a positive test tends to zero, the *predictive value* of a negative test tends to one, and vice versa. These equations also indicate that the *predictive value* is enhanced with increasing values of *sensitivity* and *specificity*. The *predictive value* of a positive test is noticeably more influenced by its *specificity* than by its *sensitivity*. The reverse is true, although to a lesser extent, for the *predictive value* of a negative test.

The equations should allow the reader to easily compute *predictive values* for variable *sensitivities*, *specificities*, and clinical response rates. Suppose, for example, that a test with a *sensitivity* of 90% and a *specificity* of 60% predicts efficacy for a patient with a drug that yields a 40% response rate in an unselected population. The probability that this patient will respond clinically to this drug is 60%.

However, the likelihood of clinical response could dramatically decrease if efficacy was found in a test with the same reliability for a less active agent or a more refractory patient population. Thus, if the expected clinical response rate in the entire population under study is 10%, the *predictive value* of a positive test becomes 20% only. With such an expected response rate, this *predictive value* would become as high as 50%, with an increase of *specificity* from 60% to 90% and an unchanged *sensitivity* of 90%.

In contrast, the *predictive value* of a negative test is extremely high in a population of patients with a disease highly resistant clinically to the therapy given. With a response rate of 10% in the entire population, the *predictive value* of a negative test will vary from 93% to 99% when *sensitivity* and *specificity* vary between 60% and 90%.

DISCUSSION

The striking relationship between the *predictive value* of a test and the expected response rate in the entire population under study has major implications. A predictive test may identify a group of patients in whom the response rate will be greater than in the entire population. However, even with a most reliable test, this improved response rate will always be dependent on the degree of therapeutic effectiveness that may be clinically expected with currently available drugs in unselected patients. The response rate to single-agent anticancer treatment varies between 0% and 30% in most diseases. A significantly increased response rate may be observed in a fraction of patients with a positive test, provided that the test has high *specificity*, but this increase will remain necessarily limited. In many cases, the advantage over empirical chemotherapy will be minimal and, in any event, the predictivity of the test will always be higher for clinical failure than for clinical response. A greater impact on cancer treatment could be expected with predictive systems for combination chemotherapy regimens, since these regimens generally yield higher response rates in unselected patients than single-agent treatment. Little experience has been accumulated in this exciting area of research which might be hampered by specific additional methodologic and technical difficulties, especially with in vitro drug testing.

These considerations are also essential when comparing various predictive tests (7). The potential of the cloning assay for the screening of new antitumor agents appears promising (8). Its value relative to conventional screening in murine models would be difficult to ascertain, since compounds lacking anticancer properties in mice are not introduced into clinical trials. Nevertheless, comparisons could be made in terms of the *predictive value* of positive tests and number of active drugs identified (9). At this point, however, insufficient information is available to estimate with accuracy the *sensitivity* and *specificity* of any test currently used to predict drug efficacy in cancer patients.

REFERENCES

1. ALBERTS DS, CHEN HSG, SOEHNLEN B, ET AL. In-vitro clonogenic assay for predicting response of ovarian cancer to chemotherapy. Lancet 2:340-342, 1980.
2. VON HOFF DD, CLARK GM, STOGDILL BJ, ET AL. Prospective clinical trial of a human tumor cloning system. Cancer Res 43:1926-1931, 1983.
3. ROZENCWEIG M, SANDERS C, PREVOST JM, ET AL. Human tumor cloning assay for the screening of new anticancer agents. Proc Am Assoc Cancer Res 23:186, 1982.
4. MAKUCH RW. Interpreting clonogenic assay results. Lancet 2:438, 1982.
5. CHIANG CL, HODGES JL, JR, and YERUSHALMY J. Statistical problems in medical diagnoses. *In* Proceedings of the Third Berkeley Symposium on Mathematical Statistics and Probability (Neyman J, ed). Berkeley, University of California Press, 1956, vol. IV, pp 121-133.

6. VECCHIO TJ. Predictive value of a single diagnostic test in unselected populations. N Engl J Med 274:1171-1173, 1966.
7. STAQUET M, ROZENCWEIG M, LEE YJ, ET AL. Methodology for the assessment of new dichotomous diagnostic tests. J Chronic Dis 34:599-610, 1981.
8. STAQUET MJ, BYAR DP, GREEN SB, ET AL. Clinical predictivity of transplantable tumor systems in the selection of new drugs for solid tumors: rationale for a three-stage strategy. Cancer Treat Rep 67:753-765, 1983.
9. SHOEMAKER RH, WOLPERT-DE FILIPPES MK, and VENDITTI JM. Application of a human tumor clonogenic assay to screening for new antitumor drugs. *In* Proceedings of the Thirteenth International Congress Chemotherapy. Vienna, 1983, vol 223, pp 14-19.

Chemosensitivity of Human Tumor Clonogenic Cells Simultaneously Assayed in Agar Diffusion Chambers and in a Two-Layer Agar Culture System[1,2,3]

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We have compared plating efficiencies (PE) of 20 fresh and cryopreserved samples of human solid tumors, disaggregated and cloned simultaneously in a two-layer agar culture system (2LACS) and in agar diffusion chambers (ADC). A significant correlation ($0.001 < P < 0.01$, paired t test) was found between PE, confirming that the two methods reflect the same property of tumor cells: their clonogenic potential. The median PE in ADC was 1.4 and 3.3 times higher than that in the 2LACS for cryopreserved and fresh specimens, respectively. One- and two-drug doses, approximating clinically achievable concentrations, were assayed simultaneously in the ADC and in the 2LACS, respectively, on 15 tumor specimens. In this way we evaluated the effects of eight drugs, two to five for each tumor, in a static (2LACS) versus a dynamic (ADC) system. The comparison of percent survival in ADC versus that in the 2LACS at both concentrations tested showed no statistically significant rank correlation. If the data regarding cyclophosphamide and mitomycin, which produced significant cell kill in the ADC and almost none in the 2LACS, were excluded, the rank correlation was still not significant. We conclude that the widely used 2LACS is unsuitable to study cyclophosphamide and mitomycin, for which the ADC technique may be a valid alternative. [Cancer Treat Rep 68:615–624, 1984]

The significant improvement of cancer chemotherapy during the last decade has allowed the development of new drugs and drug combinations with considerable clinical success (1). However, because tumors of the same origin and histologic appearance may respond quite differently to chemotherapeutic agents, suitable and reliable tests capable of measuring an individual patient's tumor sensitivity to specific drugs are necessary.

Several clonogenic and nonclonogenic methods are presently available that measure in vivo and in vitro response of human tumor cells to chemotherapy (2,3). Clonogenic assays do not always correlate well with nonclonogenic assays, but experimental evidence (2) supports the theoretical consideration that the inhibition of colony formation by tumor stem cells is the most significant end point to measure cytotoxic effect of drugs (3). Despite the great enthusiasm elicited by the introduction of a rather simple two-layer soft agar assay for human tumor clonogenic cells (4,5), this method still has major limitations: the difficulty and possible alteration of drug sensitivity of the tumor disaggregation process (6–8), "cytokinetic heterogeneity" of tumor cells (9), the low plating efficiencies (PE) of plated cells, and the artificiality of in vitro drug exposure (8,9). The complex pharmacokinetics of drugs and the in vivo interaction of the tumor with these agents and their metabolites cannot be reproduced in the test tube, and such drugs as cyclophosphamide, which need metabolic activation, cannot be evaluated without artificial activating systems, such as liver microsomal preparations. The agar diffusion chamber (ADC) technique

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(10) should theoretically provide a valid solution to some of these problems: human tumor cells in diffusion chambers implanted in the peritoneal cavity of preirradiated mice may grow better under the stimulation of diffusible growth factors present in vivo. More importantly, when drug studies are performed by this technique, the human tumor cells in the chambers are exposed to the drugs as they are metabolized by the host animal.

We report here a comparative study of eight drugs using the two-layer agar culture system (2LACS) and the ADC techniques and utilizing cell suspensions obtained from human tumors.

MATERIALS AND METHODS

Tumors.—One pleural effusion from a patient with metastatic breast carcinoma and 60 samples of solid human tumors were received directly from the operating room or the Department of Surgical Pathology at Yale-New Haven Hospital. Samples were obtained from biopsy specimens of patients who underwent surgery for diagnostic, curative, or palliative interventions. Fourteen samples were immediately processed, and 47 were stored overnight in RPMI-1640 medium with 10% fetal bovine serum (FBS) at 4°C and processed the following day. Specimens were never stored for > 24 hours before being processed.

Disaggregation procedure.—A combined sequential approach of mechanical and enzymatic disaggregation of fresh specimens was employed (11). Samples were aseptically separated from fat and tissue debris and cut with crossed scalpels. Minced tumor fragments (approximately 1 mm) were suspended in a mixture containing 0.7% collagenase II (Worthington Millipore Corp, Freehold, NJ) and 0.02% deoxyribonuclease I (Worthington) in RPMI-1640 with 10% FBS and 100 units/ml of penicillin and 100 mg/ml of streptomycin. Suspensions were incubated at 37°C for up to 5 hours with continuous mechanical agitation. They were subsequently filtered through a sterile gauze to eliminate gross cellular aggregates, passed through 25-gauge needles, washed once in RPMI-1640 with 10% FBS, and counted with a hemocytometer. Cell viability was evaluated by 0.1% trypan blue exclusion test (12), and the degree of dispersion assessed by counting the percentage of single cells, doublets, triplets, and clumps.

Two-layer agar culture system.—The method of Hamburger and Salmon (4) was modified to reflect the culture conditions of the ADC as closely as possible. A 1-ml underlayer consisting of modified (13) McCoy's 5A medium, 20% FBS (non-heat-inactivated), and 0.5% Noble agar was plated in 35-mm Petri dishes with 2-mm grid (Lux Scientific Corp, Newbury Park, CA). Heat inactivation of FBS is a definite requirement for optimal growth in the 2LACS. However, since cells in the ADC are exposed to the host serum which is not heat-inactivated, we preferred to use native FBS for a stricter comparison between the assays. Different dilutions of viable cells (10^5 to 5×10^5), depending on the number available, were suspended in a final mixture containing CMRL-1066 with 15% FBS and 0.3% Noble agar. Aliquots (1-ml) of these suspensions were plated on gelled underlayers and were incubated for 2 weeks at 37°C in 100% humidity in 7.5% CO_2. During this time, Petri dishes were routinely checked for growth twice weekly. At the end of the incubation period, colonies of ⩾ 20 cells were considered to originate from clonogenic cells and were manually counted under 40× magnification using a dissecting microscope. When the effect of continuous exposure to drugs was studied, the drugs were added to the final mixture containing the cells, plated, and left in culture for the entire period of incubation. In 1-hour exposure studies, known aliquots of cells were suspended in 4 ml of RPMI-1640 with 10% FBS containing the drug and incubated for 1 hour at 37°C. Cells were then washed twice by centrifugation (300 × *g* for 5 minutes), resuspended in CMRL-1066 and 15% FBS, and plated at the concentration indicated above.

Agar diffusion chamber assay.—The method of Smith et al (14) was followed. Chambers were filled with 10^4 to 5×10^4 viable cells (always 10% of the cell number plated in the 2LACS) and suspended in 0.35 ml of modified McCoy's 5A medium with 20% FBS and 0.3% Noble agar. After sealing with paraffin wax and gelling at room temperature for 1 hour, the chambers were surgically implanted in the peritoneal cavity of 7-10-week-old CD-1 female mice. One hour after implantation, the drugs to be tested were injected iv into the chamber-bearing animals (four to five mice per dose); control mice were injected with sterile 0.9% NaCl solution. The mice were killed 18 hours later, and the chambers were transplanted into freshly irradiated mice (660 rads of whole-body irradiation from a 250-kV Siemens machine). Two weeks later the mice were killed, the chambers were removed, and colonies of ⩾ 20 cells were counted as described above.

Cryopreservation.—Viable cells (10^6 to 5×10^6/ml) were suspended in McCoy's 5A medium containing 20% FBS and 10% dimethyl sulfoxide. Aliquots (1.8 ml) were dispensed in plastic vials, placed in a -70°C freezer for 24 hours, and then transferred to a liquid nitrogen chamber where they were stored for a maximum of 8 months before being thawed and cloned. Aliquots were thawed at 37°C and, when just thawed, washed twice with ice-cold McCoy's 5A medium with 20% FBS.

Selection of drug doses for the two assays.—The most important end point of our study was to compare in two different systems the chemosensitivity of human tumor clonogenic cells derived from the same specimens exposed to "equivalent" clinically achievable concentrations

TABLE 1.—Doses of anticancer drugs used in the 2 systems*

Drug	Human dose (mg/m^2)	Murine dose (mg/kg)	2LACS	
			High-dose (μg/ml)	Low-dose (μg/ml)
Doxorubicin	60	20	2.5	0.25
Cisplatin	60	20	2.0	0.2
Cyclophosphamide	600	200	50	5
5-FU	600	200	30	3
Methotrexate	250	83	50	5
Vinblastine	15	5	0.2	0.02
Mitomycin	30	10	1	0.1
Dacarbazine	250	83	30	3

* Modified from refs 5 and 15.

of drugs (table 1). One dose for each drug was assayed in the ADC system. The murine dose expressed in milligrams per kilogram was one-third of the human dose expressed in milligrams per square meter, since the mouse conversion factor is 3 (15). Thus, the mouse dose, on a surface area basis, approximated that given as a single dose to patients. Two concentrations for each drug were selected for the in vitro experiments. The higher dose was chosen as an approximation of the clinically achievable concentration × time values for each agent (5) and the lower was one-tenth of this. One-hour exposure was used in the 2LACS in all of the experiments except three, where continuous exposure to the same dose of drugs was tested. All drug solutions were prepared fresh on the day of experiment, and the same stocks were used for both assays.

Methods of analysis.—The survival values obtained in the two assays at different drug doses did not conform to a normal distribution. Therefore, a nonparametric method was employed: Kendall's test of rank correlation (16). The significance of the correlation between the assays can be calculated from the τ coefficient of correlation and its standard error. For comparison of PE values, which were normally distributed, both the above-mentioned test of rank correlation and the paired t test method were used.

RESULTS

Of 61 samples received, 11 were not plated due to poor yield of viable cells, 23 were cloned simultaneously in ADC and 2LACS, and the remainder were cryopreserved and/or plated in one system only. Seven cryopreserved samples were assayed in the two systems at another time; finally, cell suspensions of four specimens, which were freshly cloned and cryopreserved, were replated after thawing. In this way, direct comparison between the systems could be done in a total of 34 experiments (table 2).

Disaggregation of Tumors

The 61 specimens included 16 colon carcinomas, 14 breast carcinomas, 11 ovarian tumors, five head and neck tumors (epidermoid), four lung tumors (non-small cell), three soft tissue sarcomas, two endometrial carcinomas, two melanomas, two renal cell carcinomas, and two metastatic adenocarcinomas of unknown origin.

The median incubation time of tumor tissues with the enzymatic mixture was 2 hours and 30 minutes, with a range of 1-5 hours. The percentage of viable cells, after the disaggregation process, and their degree of dispersion are shown in tables 3 and 4, respectively. Viability was > 65% in all tumor types except for the four specimens of lung carcinoma, which were grossly more necrotic than the other samples received. The median percentage of single cells was 97.6% and that of clumps was 0%. These values reflect an excellent monocellular dispersion, although in a few cases some difficulty was encountered in the disaggregation process (range of clumps, 0%-8%).

Neither the degree of monocellular dispersion nor via-

TABLE 2.—Success of clonal growth of tumor stem cells simultaneously plated in the 2 assay systems

Samples	No. plated	No. (%) of samples showing—					
		Growth*		No growth		Contamination	
		ADC	2LACS	ADC	2LACS	ADC	2LACS
Fresh	23	14 (60)	14 (60)	6	7	3	2
Cryopreserved	11	7 (64)	7 (64)	4	4	0	0
Total	34	21 (62)	21 (62)	10	11	3	2

* Only experiments where at least 10 colonies of ≥ 20 cells/chamber or dish were considered successful. 1 fresh sample which grew in the 2LACS did not grow in the ADC; another fresh sample which grew in the ADC did not grow in the 2LACS. Therefore, only 20 samples grew in both assays.

TABLE 3.—% viability* of disaggregated tumor samples

Tumor type	No. of samples	Mean ± SD	Median	Range
Colon	16	65.0 ± 23.6	69.2	11.7-100
Breast	14	67.7 ± 14.9	65.2	50.0-100
Ovarian	11	65.9 ± 32.0	76.2	7.5-94.4
Head and neck	5	70.5 ± 26.2	75.5	36.0-100
Lung†	4	40.7 ± 39.6	37.4	0.0-88.0
Sarcoma	3	77.3 ± 15.9	85.5	59.0-87.5
Others	8	72.9 ± 9.6	69.7	59.4-89.2
Total	61	65.9 ± 24.5	70.0	0.0-100

*Viability based on trypan blue exclusion of dissociated cells prior to drug treatment and cloning.
†Non-small cell carcinomas.

bility was related to the length of exposure to the enzymatic mixture. Furthermore, viability increased fourfold after enzymatic incubation in the two samples in which viability was measured before and after incubation.

Effect of Cryopreservation on Recovery of Viable Single Cells

Table 5 summarizes the fraction of viable cells recovered after storing and thawing cryopreserved cell suspensions: on the average, one-half of the original number of cells was recovered, and the median reduction in viability approximated 40% of the original. Cryopreservation did not significantly affect the degree of monocellular dispersion.

Growth in ADC and in 2LACS

Only those experiments in which at least ten colonies of $\geq$ 20 cells were counted in control chambers or dishes were considered successful. In the case of drug studies (15 successful experiments), in only six of 30 instances was the mean number of colonies in control chambers or dishes between 10 and 30, the remaining ranging from 30 to 1513 with a median value of 144.

Table 2 shows the overall success of clonal growth for fresh and cryopreserved samples. Very similar results were observed in both systems, where approximately 60% of samples grew. The apparently better success of growth of cryopreserved cell suspensions can be explained by the selection made among the cryopreserved samples available: only those which showed the highest PE when plated fresh were selected to be recloned.

Comparison of Plating Efficiencies

A very significant correlation ($0.001 < P < 0.01$; n = 20; rank-sum tests; $0.001 < P < 0.01$; paired t test) was found between PE observed in the two assays for the specimens simultaneously cloned and grown in the ADC and in the 2LACS. The values of PE are reported in table 6 and the direct comparison is shown in figure

TABLE 4.—Degree of cellular dispersion (%)*

	Mean ± SD	Median	Range
Single cells	95.3 ± 6.4	97.6	73.3-100
Doublets	2.5 ± 4.0	1.0	0-20
Triplets	1.0 ± 1.8	0.0	0-7
Clumps	1.1 ± 1.9	0.0	0-8

*Results obtained on 60 samples of solid human tumors using a combined sequential process of mechanical and enzymatic disaggregation (*see* Materials and Methods section); hemocytometer counts.

TABLE 5.—Effects of cryopreservation on recovery of viable* single cells (%)

	No. of samples	Mean ± SD	Median	Range
Cell recovery	12	51.6 ± 24.9	53.8	12-85
Viability recovery	12	59.0 ± 28.3	57.1	15.5-117
Single-cell recovery	12	87.7 ± 11.3	90.4	58-101.8

*Viability based on trypan blue exclusion of dissociated cells prior to drug treatment and cloning.

TABLE 6.—Plating efficiencies (%) of tumor clonogenic cells assayed simultaneously in the 2 systems

Type of sample	No. of samples	2LACS			ADC		
		Mean ± SD	Median	Range	Mean ± SD	Median	Range
Fresh	13	0.17 ± 0.49	0.17	0.011-1.51	1.04 ± 1.48	0.56	0.013-5.4
Cryopreserved	7	0.17 ± 0.15	0.17	0.014-0.47	1.09 ± 2.02	0.24	0.05-5.6
Total	20	0.31 ± 0.41	0.17	0.011-1.51	1.06 ± 1.53	0.28	0.013-5.6

1. The median PE was more than three times higher in the ADC than in the 2LACS for fresh specimens, while for cryopreserved samples this difference was much less pronounced.

It was also possible to compare the PE of three specimens cloned fresh in both systems with the PE observed when the same samples were plated after cryopreservation, using the same inoculum of viable cells per chamber/dish. Median PE dropped from 0.33% to 0.05% in the 2LACS, while it dropped from 1.15% to 0.5% in the ADC.

Comparison of Drug Studies in the Two Systems

Table 7 shows the median values of percent survival of human tumor clonogenic cells observed in the two assays after drug exposure, while the direct comparison between the methods in single experiments is shown in figures 2 and 3. For clarity of graphic representation, experimental points indicating a survival > 100% of control are plotted on the line of 100% survival. This occurred five, nine, and eight times in the ADC, "2LACS low concentration," and "2LACS high concen-

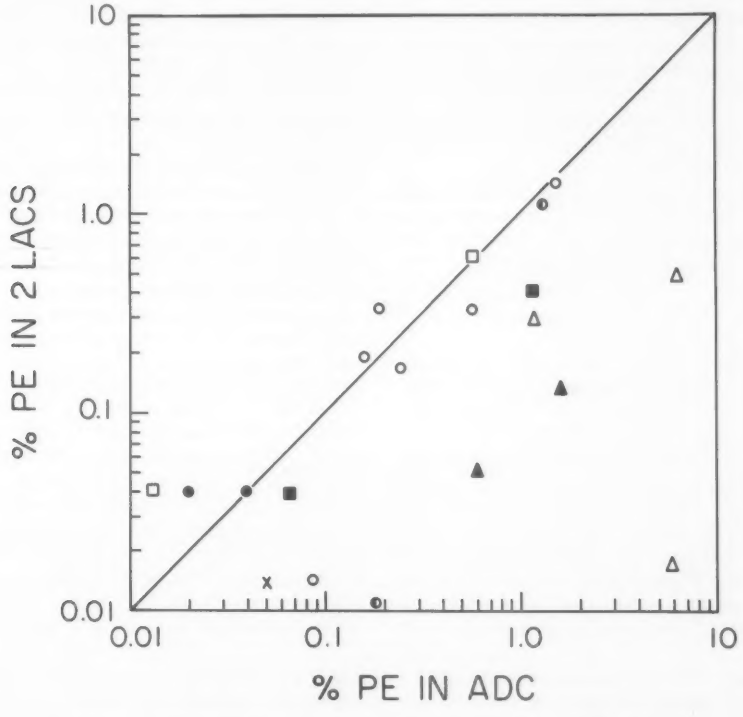

FIGURE 1.—Comparison of PE of clonogenic tumor carcinoma cells assayed in ADC and in 2LACS. ○ = ovarian carcinoma, ● = colon carcinoma, ◐ = sarcoma, △ = melanoma, ▲ = renal cell carcinoma, □ = breast cancer, ■ = endometrial carcinoma, and X = head and neck (epidermoid) carcinoma.

tration," respectively, but was never > 129.5%.

The pattern of response to methotrexate, 5-FU, doxorubicin, and dacarbazine was quite variable, with no consistently higher cell kill using either assay technique. Continuous exposure to MTX and 5-FU (three experiments) did not appear to influence the results. Interestingly, in four of 12 experiments with these two drugs the percent survival in the 2LACS low concentration was significantly lower than that found in the 2LACS high concentration.

No significant in vitro response to either concentration of vinblastine was found in three of four experiments with this drug, which induced a cell kill > 70% in one ADC experiment (fig 3).

Cisplatin and, particularly, mitomycin were more effective when tested in the ADC than in the 2LACS, where a concentration-dependent effect was evident, especially for cisplatin (fig 3).

Cyclophosphamide was ineffective in the in vitro system even when tested in high dose (50 μg/ml), but induced a cell kill > 85% in four of five specimens tested in the ADC.

Neither comparison (percent survival in the ADC vs percent survival in the 2LACS at both low and high concentrations) showed any statistically significant correlation. It was remarkable that if the data regarding mitomycin and cyclophosphamide were excluded, the rank correlation was still not significant. In none of the 12 tumors where ⩾ 70% cell kill was observed in the ADC was there an equivalent kill by either concentration of drug tested in the 2LACS (figs 2 and 3).

The correlation between the two concentrations tested in the 2LACS was high ($0.001 < P < 0.01$; n = 23); a significantly lower cell survival ($0.001 < P < 0.01$; n = 38; paired t test) was found at the high concentration.

DISCUSSION

Direct comparisons of in vitro and in vivo clonogenic methods to study growth and chemosensitivity of mammalian tumor cells have been reported in several studies (3,17,18) in the 26 years since clonal growth of mammalian cells was first observed (19). What is unique to our study is that our comparison was done simultaneously, using cell suspensions derived from the disaggregation of fresh human tumor specimens obtained directly at surgery. Because of the disadvantages mentioned in the Introduction, the widely used 2LACS needs improvement (6,8,10). In this connection, we undertook the present investigation to answer two basic questions: (*a*) do human clonogenic tumor cells grow better in the "quasi in vivo" system of ADC (20) than in vitro? and (*b*) do the two assays correlate in terms of drug sensitivity?

Our results of the disaggregation procedure compare well with those of other investigators with regard to viability (6,12). We also report a quantitation of the degree of monocellular dispersion in cell suspensions after processing the samples. The percentage of single cells was higher than that obtained by Drewinko et al (21) in their study of the optimization of enzymatic collection and dispersion of a human colonic carcinoma cell line growing in monolayer culture. This type of quantitation for fresh human tumors has not previously been reported to our knowledge and may be very important. In fact, the production of a good single-cell suspension is an essential requisite of clonogenic assays; the greater tendency of cells in clumps to grow in culture (8), phenomena like the "clonogenic clusters" (22), or simply erroneous reading of clumps as "colonies" by image analyzers may seriously affect the results derived from these assays. Even if we did not achieve a "pure" single-cell suspension, the effects of these possible sources of error were minimized in our study by the production of a high degree of monocellular dispersion and by the manual count of Petri dishes and chambers, which warrants a sensitive discrimination between colonies and plated clumps.

Cryopreservation is an excellent technique for additional studies of cell biology and chemosensitivity of tumors. Our data indicate that a large number of fresh, viable cells (in the range of 10^8) are needed for cryopreserva-

TABLE 7.—% survival after drug exposure

Drug	No. of samples	ADC		2 LACS			
				Low-concentration		High-concentration	
		Median	Range	Median	Range	Median	Range
Cyclophosphamide	5	8.6	2.6-70.8	104.8	92.6-116.7	96.4	90.2-115.1
Mitomycin	5	26.5	5.1-35.4	82.1	70.5-97.5	67.8	30.1-77.7
Vinblastine	4	75.9	13.9-108.7	99.5	69.4-113.9	96.0	71.5-101.8
Cisplatin	6	44.5	5.6-75.2	93.0	71.2-124.7	66.4*	18.2-114.3
Doxorubicin	5	87.7	21.6-112.5	86.8	54.5-121.4	76.5†	10.6-116.7
5-FU	6	65.0	39.0-98.0	88.8	29.6-94.0	70.0	35.4-104.8
Methotrexate	6	82.4	53.3-116.6	90.6	23.6-101.4	94.3	41.4-114.6
Dacarbazine	5	91.0	3.3-110.0	93.1	76.3-129.5	93.9	53.2-101.2

* Median of 5 samples only.
† Median of 4 samples only.

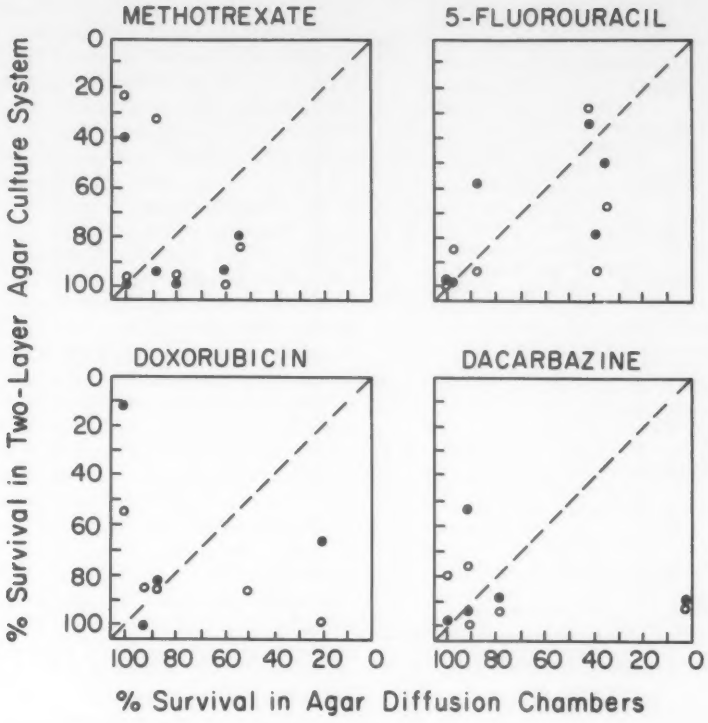

FIGURE 2.—% survival of clonogenic tumor cells exposed to methotrexate, 5-FU, doxorubicin, and dacarbazine in the 2 systems. Same cell suspensions were used for both assays. Cells, plated in the ADC implanted in the peritoneal cavity of mice, were exposed to 1 dose of drug given iv to host animals, while in the 2LACS, cell suspensions were incubated in vitro for 1 hr with 2 concentrations of drug before cloning. 2 of the MTX experiments and 1 of the 5-FU experiments in the 2LACS were done using continuous drug exposure. Results are expressed as % survival of untreated control. ○ = the comparison of ADC vs 2LACS low-drug concentration; ● = the comparison of ADC vs 2LACS high-drug concentration.

tion without programmed freezing because, on the average, only one-fourth of the original number of viable cells were recovered after thawing. A further 50%–80% reduction in clonogenicity of cryopreserved cells in either system made the repetition of experiments possible with only very few samples. Selby and Steel (23) did not find a decrease in clonogenic potential of cell suspensions of human tumors in the ADC system after cryopreservation using programmed freezing. Despite the very significant reduction in viability, our study indicates that programmed freezing is not an absolute requirement to retain viability.

The greater PE of human tumor cells which we found in the ADC, as compared to the 2LACS, may be explained by the continuous replenishment of fresh nutrients and growth factors provided by the host mouse, but it remains very difficult to explain why such a difference was not as marked for cryopreserved samples as it was for fresh specimens. In a similar study, Courtenay et al (20) compared PE of fresh human tumor cells cloned in ADC and in test tubes using soft agar with a replenishable liquid phase and found no difference between PE in the two assays. The future application of refeeding systems to the 2LACS may provide partial improvement of PE. Other possible explanations for the greater PE in the ADC include different media and a slightly different cell concentration. The high degree of statistical correlation among PE in the two systems confirms that these reflect the same property of tumor cells: their clonogenic potential.

In general, chemosensitivity of human tumor cells was more marked with the ADC assay. This method might have produced a greater inhibition of colony growth simply because an exposure time longer than that used in vitro was employed (18 hours vs 1 hour). Despite our attempt to expose the cell suspensions in the two systems

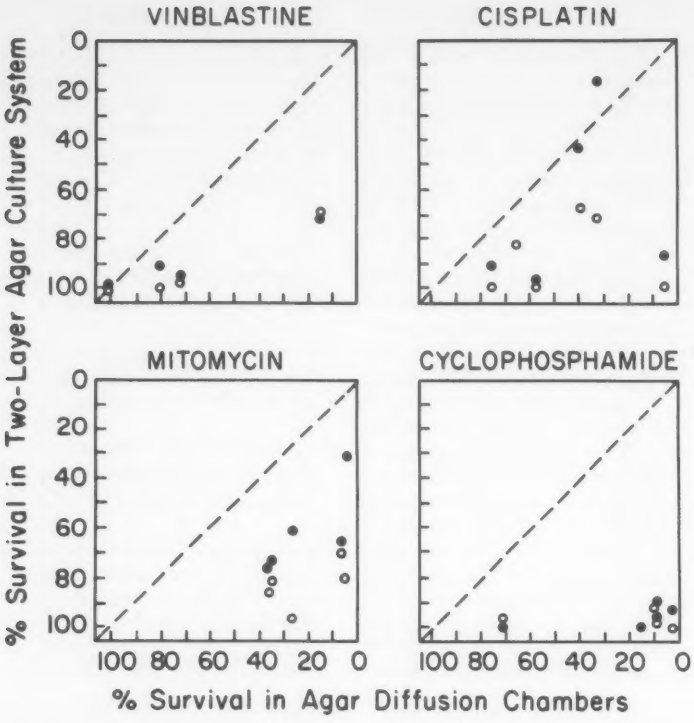

FIGURE 3.—% survival of clonogenic tumor cells exposed to vinblastine, cisplatin, mitomycin, and cyclophosphamide in the 2 systems. Same cell suspensions were used for both assays. Cells, plated in ADC implanted in the peritoneal cavity of mice, were exposed to 1 dose of drug given iv to host animals, while for the 2LACS, cell suspensions were incubated in vitro for 1 hr with 2 concentrations of drug before cloning. Results are expressed as % survival of untreated control. ○ = comparison of ADC vs 2LACS low-drug concentration; ● = comparison of ADC vs 2LACS high-drug concentration.

to concentrations × time which are clinically achievable, we must emphasize the potential for significant differences in the drug concentrations in the dynamic system (ADC) versus those obtained in the static system (2LACS). In addition, very little is known about drug penetration into the ADC. Selby and Steel (24) showed that the serum level of [^{14}C]melphalan was higher than that in the ADC during the entire period of observation (2 hours). On the other hand, the opposite result was obtained by Ambrose and Lowrey (25) using *cis*- or *trans*-[^{195m}Pt]dichlorodiammineplatinum. The latter study was done using diffusion chambers without agar. Nonetheless, the activity of cisplatin we found in the ADC may be due to a higher than expected level of this drug in the chambers. The significant decrease in survival found in the 2LACS high concentration as compared to the ineffectiveness of this drug at low concentration indicates that cisplatin is active in both systems but higher concentrations must be tested in vitro to achieve significant cytotoxicity. The ineffectiveness of cyclophosphamide in the 2LACS was expected (26), since this drug must be activated by the liver to exert a cytotoxic effect (27). In vivo activity of mitomycin is also due to its enzymatic reductive activation (28), which may explain the more marked sensitivity of cells in the ADC. In addition, the cytotoxicity of this drug has been shown to be greater under hypoxic as compared to oxic conditions (29). The oxygen tension inside the intraperitoneal chambers may not be as high as that in the Petri dishes incubating in 92.5% air. The nonphysiologic, light-dependent activation of dacarbazine to diazoimidazole carboxamide (30) might have been responsible for the inconsistent cytoxicity of this drug in both assays. The other four drugs never gave a 90% reduction in colony number with either method, even at the highest concentration tested. This apparent insensitivity can be explained by: (*a*) natural resistance of tumors to the drugs tested; (*b*) pharmacologically inappropriate drug exposure with respect to biotransforma-

tion, concentration, and time; and (c) protection of cells from the effects of antimetabolites such as methotrexate (31,32) and 5-FU (33,34) by undialyzed FBS containing significant amounts of nucleosides and folates. In eight additional experiments using the 2LACS only, we found MTX inactive even when continuous exposure to 5 and 50 μg/ml of drugs was tested (A. F. Sobrero, J. C. Marsh, and J. R. Bertino, unpublished data).

The different sensitivity of human clonogenic cells to the same drugs assayed in the two systems may be explained by the above considerations. In addition, the use of a strict definition of colony size (35) and higher minimum colony numbers in control dishes or chambers (36) may have given a more accurate comparison, but the number of successful experiments would be decreased significantly because of the limited number of tumor cells available and their low PE. The most important result of our study is the lack of correlation between the assays found in the drug studies as opposed to the strong positive correlation of PE. None of the patients whose tumor specimen was assayed in our systems had had prior chemotherapy and only three were subsequently given chemotherapy with some of the agents we studied; for the latter, it is too early to evaluate the clinical response. Therefore, clinical correlations, which are the only way to verify the predictive power of the tests, are not yet available. Several authors have reported that when the 2LACS is used as a chemosensitivity test, the results correlate well with clinical response: 94%–98% true-negative for resistance and 60%–70% true-positive for sensitivity (5,37,38). The ADC has also been used to predict drug sensitivity of individual patients' tumor specimens (39) and the results are comparable. Our data indicate that the 2LACS is not applicable to study cyclophosphamide and mitomycin, for which the ADC technique is a valid alternative. This procedure is expensive and more time-consuming than the 2LACS, but at present we suggest that it may be the assay of choice for cyclophosphamide and mitomycin and that it is a useful complement to the 2LACS in the study of agents where in vivo drug metabolism is important.

REFERENCES

1. DEVITA VT, JR. Principles of chemotherapy. *In* Cancer. Principles and Practice of Oncology (DeVita VT, Jr, Hellman S, and Rosenberg SA, eds). Philadelphia, Lippincott, 1982.
2. ROPER PR, and DREWINKO B. Comparison of in vitro methods to determine drug-induced cell lethality. Cancer Res 36:2182–2188, 1976.
3. STEEL GG, ed. Growth Kinetics of Tumours. Oxford, Clarendon Press, 1977.
4. HAMBURGER AW, and SALMON SE. Primary bioassay of human tumor stem cells. Science 197:461–463, 1977.
5. SALMON SE, ed. Cloning of Human Tumor Stem Cells. New York, Alan R. Liss, 1980.
6. MACKINTOSH FR, EVANS TL, and SIKIC BI. Methodologic problems in clonogenic assays of spontaneous human tumors. Cancer Chemother Pharmacol 6:205–210, 1981.
7. RASEY J, and NELSON N. Effect of tumor disaggregation on results of in vitro cell survival assay after in vivo treatment of the EMT6 tumor: X-rays, cyclophosphamide and bleomycin. In Vitro 16:547–553, 1980.
8. ROSENBLUM ML, DOUGHERTY DV, REESE C, ET AL. Potentials and possible pitfalls of human stem cell analysis. Cancer Chemother Pharmacol 6:277–235, 1981.
9. FREI E, and LAZARUS H. Predictive tests for cancer chemotherapy. N Engl J Med 298:1358–1359, 1978.
10. GORDON MY. Quantitative of haemopoietic cells from normal and leukaemic RFM mice using an in vivo colony assay. Br J Cancer 30:421–428, 1974.
11. SLOCUM HK, PAVELIC ZP, KANTER PM, ET AL. The soft agar clonogenicity and characterization of cells obtained from human solid tumors by mechanical and enzymatic means. Cancer Chemother Pharmacol 6:219–225, 1981.
12. TENNANT JR. Evaluation of the trypan blue technique for determination of cell viability. Transplantation 2:685, 1964.
13. PIKE BL, and ROBINSON WA. Human bone marrow colony growth in agar-gel. J Cell Physiol 75:77–84, 1970.
14. SMITH IE, COURTENAY VD, and GORDON MY. A colony forming assay for human tumour xenografts using agar in diffusion chambers. Br J Cancer 34:476–483, 1976.
15. FREIREICH EJ, GEHAN EA, RALL DP, ET AL. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219–244, 1966.
16. SWINSCOW TDV. Statistics at Square One. London, Dawson and Goodall Ltd, 1976.
17. BLACKETT NM, MARSH JC, GORDON MY, ET AL. Simultaneous assay by six methods of the effect on haemopoietic precursor cells of adriamycin, methyl-CCNU, ^{60}Coγ-rays, vinblastine and cytosine arabinoside. Exp Hematol 5:2–8, 1978.
18. STEEL GG, and ADAMS K. Stem cell survival and tumor control in the Lewis lung carcinoma. Cancer Res 35:1530–1535, 1975.
19. PUCK TT, MARCUS PI, and CIECIURA SJ. Clonal growth of mammalian cells in vitro. J Exp Med 103:273–283, 1956.
20. COURTENAY VD, SELBY PJ, SMITH TE, ET AL. Growth of human tumour cell colonies from biopsies using two soft-agar techniques. Br J Cancer 38:77–81, 1978.
21. DREWINKO B, ROMSDAHL MM, YANG LY, ET AL. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. Cancer Res 36:467–475, 1976.
22. TISMAN G, and WU SJG. The "clonogenic cluster": an interesting laboratory observation. Proc Am Assoc Cancer Res and ASCO 22:236, 1981.
23. SELBY PJ, and STEEL GG. Clonogenic cell survival in cryopreserved human tumour cells. Br J Cancer 43:143–148, 1981.
24. SELBY PJ, and STEEL GG. The use of the agar diffusion chamber for the exposure of human tumor cells to drugs. Cancer Res 42: 4758–4762, 1982.
25. AMBROSE KR, and LOWREY JS. Effects of *cis*- and *trans*-dichlorodiammineplatinum(II) on human tumor cell proliferation in diffusion chambers in vivo. Cancer Res 42:1769–1773, 1982.
26. ARNOLD H, BOURSEAUX F, and BROCK N. Chemotherapeutic action of a cyclic nitrogen mustard phosphamide ester (B518-ASTA) in experimental tumours of the rat. Nature 181:931, 1958.
27. BROCK N. Pharmacologic characterization of cyclophosphamide (NSC-26271) and cyclophosphamide metabolites. Cancer Chemother Rep 51:315–325, 1967.
28. KENNEDY KA, and SARTORELLI AC. Metabolic activation of mitomycin C by isolated liver nuclei and microsomes. Fed Proc 38:443, 1979.
29. TEICHER BA, LAZO JS, and SARTORELLI AC. Classification of antineoplastic agents by their selective toxicities toward oxygenated and hypoxic tumor cells. Cancer Res 41:73–81, 1981.
30. BONO VH. Studies on the mechanism of action of DTIC (NSC-45388). Cancer Treat Rep 60:141–148, 1976.
31. NEUMAN D, and TISMAN G. Attempts to quality control and standardize the human tumor stem cell assay (HTSCA). Clin Res 30:70, 1982.
32. PINEDO HM, ZAHARKO DS, BULL JM, ET AL. The reversal of metho-

trexate cytotoxicity to mouse bone marrow cells by leucovorin and nucleosides. Cancer Res 36:4418–4424, 1976.
33. DREWINKO B, YANG LY, HO DH, ET AL. Treatment of cultured human colon carcinoma cells with fluorinated pyrimidines. Cancer 45:1144–1158, 1980.
34. JONES HM, and BRUCE WR. On the mechanism of the lethal action of 5-fluorouracil on mouse L cells. Cancer Res 28:1976–1981, 1958.
35. NIAS AHW, and FOX M. Minimum clone size for estimating normal reproductive capacity of cultured cells. Br J Radiol 41:468–474, 1968.
36. BLACKETT NM. Statistical accuracy to be expected from cell colony assays; with special reference to the spleen colony assay. Cell Tissue Kinet 7:407–412, 1974.
37. SALMON SE, HAMBURGER AW, SOEHNLEN BJ, ET AL. Quantitation of differential sensitivity of human tumor stem cells to anti-cancer drugs. N Engl J Med 298:1321–1327, 1978.
38. VON HOFF DD, CASPER J, BRADLEY E, ET AL. Association between human tumor colony-forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 70:1027–1032, 1981.
39. MARSH JC, and KIRKWOOD JM. The in vivo agar diffusion chamber (ADC) for melanoma drug sensitivity assay: clinical correlations. Stem Cells 1:321, 1982.

Relationship Between the Induction of Chromosome Damage and Cytotoxicity for Amsacrine and Congeners[1,2]

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The antitumor drug amsacrine causes chromosomal aberrations both in cultured cells (L1210 murine leukemia and HeLa cells) and in vivo (L1210 leukemia). The proportions of different types of aberrations, mainly chromatid gaps, chromatid exchanges, and acentric chromosome fragments, are similar in both the in vivo and in vitro experiments. A small proportion of cells, particularly after drug exposure for 24 hours, show extensively damaged or pulverized chromosomes. In in vitro experiments using HeLa cells and amsacrine together with two analogs of amsacrine with differing cytotoxic activity, an inverse relationship between the frequency of chromosomal aberrations and cell survival has been demonstrated. The results are consistent with the proposal that for this class of drugs, the induction of chromosomal aberrations, both in vivo and in vitro, leads to cell death. [Cancer Treat Rep 68:625–630, 1984]

Many derivatives of 4′-(9-acridinylamino)methanesulfonanilide (AMSA) display high, broad-spectrum experimental antitumor activity (1), and one of the more dose-potent analogs (amsacrine or *m*-AMSA; fig 1) has undergone clinical trial (2). A detailed understanding of the mode of cytotoxic action of *m*-AMSA is an important prerequisite for the rational design of more active agents.

m-AMSA binds to DNA by intercalation (3,4), and it seems likely that cellular DNA is the site of action. Cytogenetic studies have shown that *m*-AMSA causes sister chromatid exchange in phytohemagglutinin-stimulated blood lymphocytes (5) and chromosomal aberrations in cultured Chinese hamster (6) and L1210 (7) cells.

We have analyzed the chromosomes of cells exposed in vivo and in vitro to various concentrations of *m*-AMSA. To answer the question of whether chromosomal aberrations lead to cell death, we have determined the relationship between cell survival (determined using a clonogenic assay) and incidence of chromosomal aberrations for *m*-AMSA and two of its analogs.

MATERIALS AND METHODS

Drugs.—The AMSA analogs were used as the chloride salts. Details regarding chemical synthesis have been published previously (8); samples were homogeneous as judged by thin-layer chromatography. Stock solutions of drug (2 mg/ml) were prepared in 50% ethanol and stored at 4°C in the dark.

Cell lines.—HeLa cells were obtained from Professor A. R. Bellamy, Department of Cell Biology, Auckland University, and were maintained in TC199 medium, supplemented with 10% neonatal calf serum (Gibco, New Zealand), at 37°C in an atmosphere of 5% CO_2 and 95% air. L1210 cells were initially obtained from the National Cancer Institute (Bethesda, MD), and were maintained in DBA/2J mice, stocks of which were initially obtained from The Jackson Laboratory in the United States (cf, ref 1).

Examination of chromosome damage in vivo.—Mice were inoculated with L1210 leukemia (10^5 cells/mouse) ip on Day 0. On Day 2, groups of mice were injected ip with cytotoxic doses of *m*-AMSA (5 or 10 mg/kg), and both treated and untreated groups were injected with

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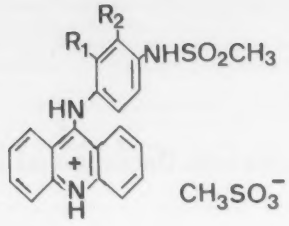

AMSA : $R_1 = R_2 = H$

m-AMSA : $R_1 = OCH_3$ $R_2 = H$

o-AMSA : $R_1 = H$ $R_2 = OCH_3$

FIGURE 1.—Structures of derivatives of 9-anilinoacridine.

vinblastine (1.5 mg/kg) either 2 or 24 hours after this time. Two hours later, the mice were killed by cervical dislocation and the L1210 cells were aspirated from the peritoneal cavity. Chromosomes were harvested and stained by standard procedures (9).

Examination of chromosome damage in vitro.—L1210 cells were harvested from mice and maintained in tissue culture for at least 3 weeks before use in experiments (10). Cells in logarithmic phase were diluted fourfold and grown for 16 hours before exposure to drug for 2 hours. HeLa cells were trypsinized, plated at 20,000 cells/60-mm Petri dish, and grown for 16 hours before exposure to a range of drug concentrations for 2 hours. For both cell types, cells were subsequently washed with phosphate-buffered saline (PBS); fresh, drug-free medium containing 0.06 μg/ml of colchicine was then added, and the cells were grown for 4 hours (HeLa) or 2 hours (L1210), harvested, and stained with Giemsa using standard procedures. Fifty mitotic figures were scored for varying categories of chromosome damage, and the results were expressed as the percentage of cells possessing aberrant chromosomes. A minimum of 5000 cells/slide were scored to provide the percentage of cells in metaphase, and the mitotic index was expressed as the ratio of this figure to the value for untreated control.

Clonogenicity assays.—HeLa cell cultures were trypsinized, washed with PBS, and aspirated several times to produce a single-cell suspension. Cells were plated at a density of 200 or 2000 cells/60-mm Petri dish, fresh medium was added, and 6 hours later, a range of drug doses were added to the dishes. After 2 hours, cells were washed with PBS and fresh medium was added. Plates were incubated for 1 additional week, with one medium change on the 4th day. Colonies were fixed and stained with Giemsa before scoring.

RESULTS AND DISCUSSION

Comparison of Chromosomal Aberrations in L1210 Cells Treated With *m*-AMSA In Vivo and In Vitro

Doses of *m*-AMSA in the therapeutic range (1) were administered ip to mice and produced a variety of chromosomal aberrations within 2 hours of treatment (table 1). Chromatid gaps were the most frequently observed aberration, followed by chromatid interchanges and acentric chromosome fragments. A similar frequency of chromosomal aberrations and a similar distribution of different types of aberration were produced by in vitro exposure of cultured L1210 cells to concentrations of *m*-AMSA slightly above (10) those causing 50% inhibition of growth. A lower mitotic index was observed in vivo than in vitro after 2 hours, probably reflecting that the intraperitoneal *m*-AMSA concentration is initially higher than that used in vitro. The mitotic index increased to about one-third of the control value by 24 hours after drug injection (table 1), presumably indicating a recovery of G2-phase growth arrest (6) following the disappearance of *m*-AMSA from the circulation.

Appearance of "Pulverized" Chromosomes

In the in vivo experiments a significant proportion of L1210 cells harvested 24 hours after *m*-AMSA treatment showed more extensive chromosome derangement (fig 2, a and b). One example was also noted in L1210 cells harvested 2 hours after drug exposure (table 1). There appeared to be two types of aberration.

In the first type only a small proportion of chromosomes were affected, in a restricted area of the cell. They were stippled (fig 2a) and were similar in appearance to those described by Johnson and Rao (11) in virus-fused cells. In the second type (fig 2b) all chromosomes were affected, and the cells showed extreme chromosome fragmentation. Both types of aberration have previously been reported in cells treated by the antitumor drug bleomycin (12).

Effect of *m*-AMSA on HeLa Cell Chromosomes In Vitro

HeLa cells were exposed to a range of concentrations of *m*-AMSA and were scored for mitotic index and incidence of chromosomal aberration. In a parallel experiment, surviving cells were determined using a clonogenic assay (fig 3, a and b). The number of cells with aberrant chromosomes increased as a function of dose, showing a reciprocal relationship with mitotic index. Cell survival decreased logarithmically with dose, showing little or no shoulder in the survival curve. The types of chromosomal aberrations were similar to those

TABLE 1.—Chromosome aberrations produced by *m*-AMSA in L1210 cells

	In vivo					In vitro		
	Control	5 mg/kg of *m*-AMSA 24 hrs before	10 mg/kg of *m*-AMSA 24 hrs before	5 mg/kg of *m*-AMSA 2 hrs before	10 mg/kg of *m*-AMSA 2 hrs before	Control	40 ng/ml of *m*-AMSA 2 hrs before	80 ng/ml of *m*-AMSA 2 hrs before
Mitotic index (metaphase/10,000 cells)	100	39.49	31.75	15.16	2.05	100	31.20	26.05
% cells with aberrant metaphase	8	28	44	42	48	2	38	46
Total No./100 cells								
Chromatid gaps	6	4	8	46	58	4	42	54
Chromatid breaks	0	0	2	6	14	0	6	8
Chromatid interchanges	0	0	4	18	28	0	22	24
Dicentric or ring chromosomes	0	4	6	4	2	0	0	2
Acentric chromosome fragments	6	36	58	10	22	0	12	26
Premature chromosome condensation or pulverized chromosomes	0	14	8	0	2	0	0	0

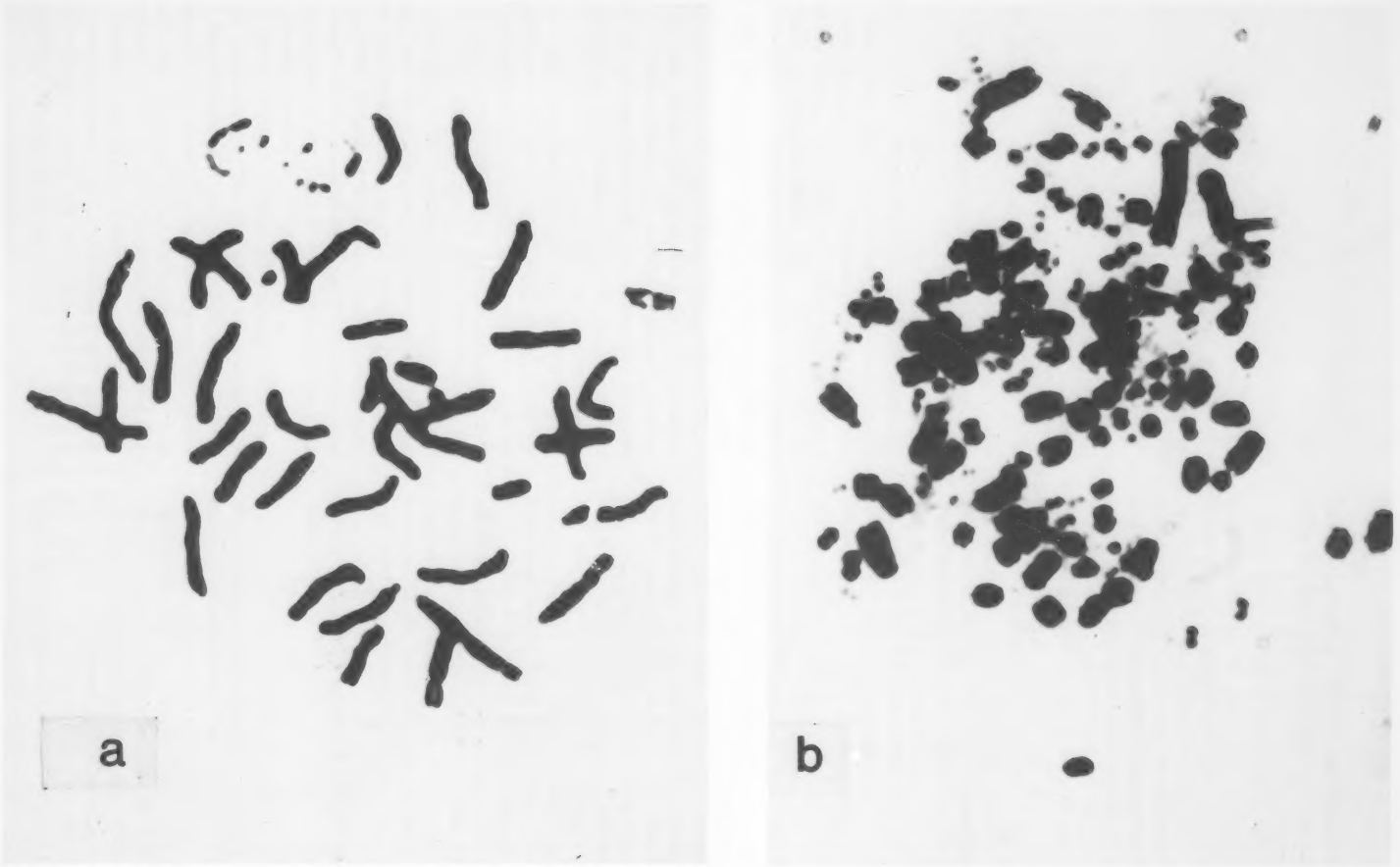

FIGURE 2.—Chromosome spreads from L1210 cells treated in vivo with *m*-AMSA. Leukemia-bearing mice were dosed with *m*-AMSA (10 mg/kg). After 24 hrs, they were killed by cervical dislocation, and cells were aspirated from the intraperitoneal cavity for chromosomal analysis. a. Most chromosomes appear essentially normal, except for 2 ring chromosomes, both of which are prematurely condensed. b. Heavily damaged cell, with pulverized chromosomes.

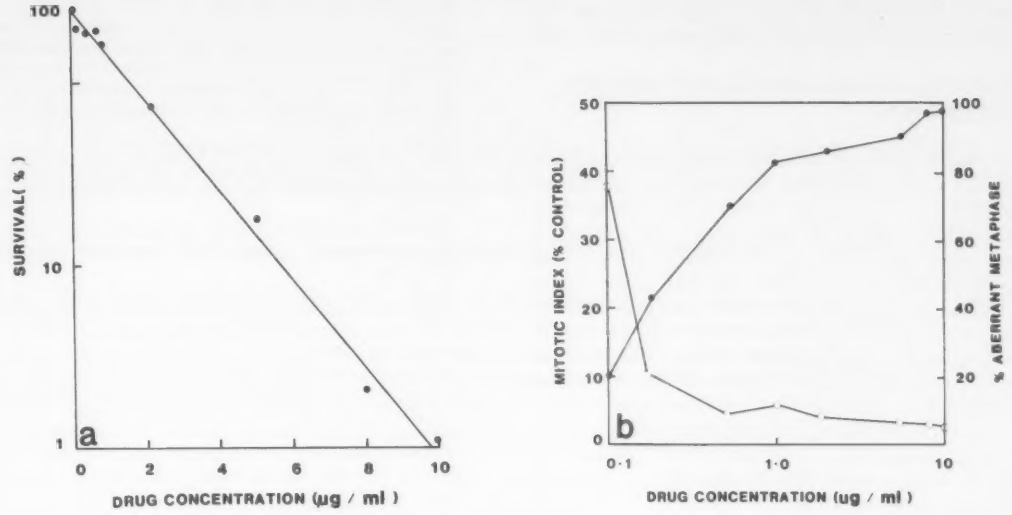

FIGURE 3.—Colony survival (a) and chromosome aberrations (●) and mitotic index (○) (b) of HeLa cells, after exposure to *m*-AMSA. Exponentially growing cells were exposed to the drug for 2 hrs and were washed 3 times with PBS. Fresh medium was added and the cells were grown for 10 days before scoring for surviving colonies (a). In some cultures, colchicine (0.06 µg/ml) was added and cells were grown for 4 hrs before harvesting and scoring (b).

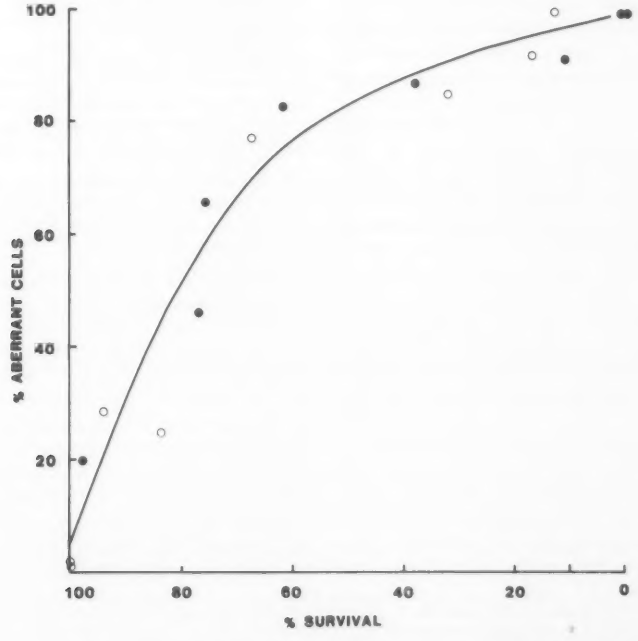

FIGURE 4.—Relationship between survival (as measured by the ability to form colonies) and % of mitotic HeLa cells with aberrant chromosomes for *m*-AMSA (●) and AMSA (○).

observed with L1210 cells, and at high drug doses, several examples of "pulverized" chromosomes were noted.

Relationship Between Chromosomal Aberrations and Cytotoxicity

To extend the range of values in the relationship discernible in figure 3, two less active congeners of *m*-AMSA were tested (fig 1). The first of these structures (AMSA) lacks a methoxy group, and although it is an active antitumor agent, it is approximately sixfold less dose-potent than *m*-AMSA (8). The second analog (*o*-AMSA) is a positional isomer with the methoxy group *ortho* to the methanesulfonamide group. It is inactive towards L1210 leukemia (8) and caused no increase in chromosomal aberrations over the whole dose range. Data for the other two compounds are plotted in figure 4. All points lie on the same curve, indicating a common relationship. Cell survival of 50% is associated with an approximately 85% incidence of chromosomal aberration in mitotic cells.

CONCLUSIONS

The results demonstrate that *m*-AMSA, administered either in vivo or in vitro in low doses, produces the same types of chromosomal aberrations. *m*-AMSA is known to be metabolized in the liver (13), but its effectiveness in vitro, together with the results described above, suggests that liver metabolism is not required for activity. The inverse relationship between cell survival and incidence of chromosomal damage, demonstrated for derivatives of 9-anilinoacridine with varying degrees of antitumor activity, is consistent with the hypothesis that chromosomal damage leads to cell death. This damage may well be the first microscopically visible consequence of DNA breakage which is now well-documented as an early consequence of cellular exposure to *m*-AMSA (14-17).

REFERENCES

1. CAIN BF, and ATWELL GJ. The experimental antitumour properties of three congeners of the acridinylmethanesulphonanilide (AMSA) series. Eur J Cancer 10:539-549, 1974.
2. LEGHA SS, GUTTERMAN JN, HALL SW, ET AL. Phase I clinical investigation of 4'-(9-acridinylamino) methanesulfon-*m*-anisidide (NSC 249, 992), a new acridine derivative. Cancer Res 38:3712-3716, 1978.
3. WARING MJ. DNA binding characteristics of acridinyl methanesulphonanilide drugs: comparison with antitumour properties. Eur J Cancer 12:995-1001, 1974.
4. WILSON WR, BAGULEY BC, WAKELIN LPG, ET AL. Interaction of the antitumour drug 4'-(9-acridinylamino) methanesulfon-*m*-anisidide and related acridines with nucleic acids. Molec Pharmacol 20:404-414, 1981.
5. CROSSEN P. The effect of acridine compounds on sister chromatid exchange formation in cultured human lymphocytes. Mutat Res 68:295-299, 1978.
6. DEAVEN LL, OKA MS, and TOBEY RA. Cell cycle specific chromosome damage following treatment of cultured Chinese hamster cells with 4'-[(9-acridinyl)-amino] methanesulphon-*m*-anisidide HCl. JNCI 60:1155-1159, 1971.
7. BAGULEY BC, WILSON WR, FERGUSON LR, ET AL. Analysis of acridinylaminomethanesulphonanilide derivatives, using DNA binding and other assays to predict experimental antitumour activity. *In* Current Chemotherapy (Siegenthaler W, and Luthy R, eds). Washington, DC, American Society for Microbiology, 1978, pp 1210-1212.
8. CAIN BF, ATWELL GJ, and DENNY WA. Potential antitumour agents, 16. 4'-(Acridin-9-ylamino)-methanesulfonanilides. J Med Chem 18:1110-1117, 1975.
9. HAYFLICK L, and MOORHEAD PS. The serial cultivation of human diploid cell strains. Exp Cell Res 25:585-621, 1961.
10. BAGULEY BC, and NASH R. Antitumour activity of substituted 9-anilinoacridines—comparison of in vivo and in vitro testing systems. Eur J Cancer 17:671-679, 1981.
11. JOHNSON RT, and RAO PN. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. Nature 226:717-722, 1970.
12. IKEUCHI T. Chromosome pulverization in Chinese hamster multinucleate cells induced by bleomycin and cytochalasin B. Jpn J Genet 48:391-405, 1973.
13. SHOEMAKER DD, CYSYK RL, PADMANABHAN S, ET AL. Identification of the principal biliary metabolite of 4'-(9-acridinylamino)methanesulfon-*m*-anisidide in rats. Drug Metab Dispos 10:35-39, 1982.
14. FURLONG NB, SATO J, BROWN T, ET AL. Induction of limited DNA damage by antitumour agent Cain's acridine. Cancer Res 38:1329-1335, 1978.
15. RALPH RK. On the mechanism of action of 4'-[(9-acridinyl)-amino] methanesulphon-*m*-anisidide. Eur J Cancer 16:595-600, 1980.
16. ZWELLING LA, KERRIGAN D, MICHAELS S, ET AL. Cooperative sequestration of *m*-AMSA in L1210 cells. Biochem Pharmacol 31:3269-3277, 1982.
17. ZWELLING LA, MICHAELS S, ERICKSON LC, ET AL. Protein-associated deoxyribonucleic acid strand breaks in L1210 cells treated with the deoxyribonucleic acid intercalating agents 4'-(9-acridinylamino) methanesulfon-*m*-anisidide and Adriamycin. Biochemistry 20:6553-6563, 1980.

Abnormal Deoxyuridine Suppression as a Rapid Predictor for Antimetabolite Chemosensitivity: Correlation With In Vitro Tests of Growth Inhibition[1]

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The deoxyuridine (dU) suppression test, which estimates the activity of the de novo pathway to DNA synthesis from dU, was evaluated as a predictor of antimetabolite growth inhibition. Observations of growth inhibition were made using flask cell culture and soft agar clonogenic assay and correlated with results of the rapidly performed dU suppression test in human (SK-L7) leukemia cells, in methotrexate-sensitive and -resistant murine (L1210) leukemia cells, and in human tumor explants. The concentration of methotrexate resulting in a positive dU suppression test was closely correlated with the methotrexate concentrations required for growth inhibition in flask and soft agar culture systems. The fact that the dU suppression test can be rapidly interpreted in 4 hours compared to the longer period required for clonogenic assay suggests that further evaluation of this procedure as a rapid predictor for clinical antimetabolite response is warranted. [Cancer Treat Rep 68:631–636, 1984]

Herbert and co-workers (1) observed that folate or vitamin B_{12} deficiency causing clinical megaloblastosis could be measured in short-term bone marrow cultures using the deoxyuridine (dU) suppression test. This test reflects the extent to which added dU suppresses the incorporation into DNA of ^{3}H-thymidine or its gamma-emitting analog, ^{125}I-iododeoxyuridine. In the presence of adequate folate or B_{12}, unlabeled dU added in excess results in production of dTMP and consequent failure of labeled thymidine to be incorporated into DNA-thymine. Depletion of folate or B_{12}, with associated thymidylate synthetase inhibition, resulted in relative inability to form dTMP from dU and consequently increased incorporation of labeled thymidine into DNA. The dU suppression test has, therefore, been used to assess inhibitory influences on the de novo pathway of DNA-thymine synthesis from dU (1,2).

Both methotrexate and 5-FU have known inhibitory effects on thymidylate synthetase and could be expected to have an influence on the dU suppression test which is similar to the influence of folate or vitamin B_{12} deficiency. Methotrexate inhibits dihydrofolate reductase which is responsible for converting dihydrofolate to tetrahydrofolate, thus disturbing the methylation of dUMP by decreasing the pool of 5,10-methylene tetrahydrofolate (3). 5-FU acts as a direct inhibitor of thymidylate synthetase (4) following its activation to 5-FdUMP. Based on the similar effects of folate or B_{12} deficiency and of administration of 5-FU or methotrexate on thymidylate synthetase, we examined the ability of the rapidly performed dU suppression test to predict antimetabolite growth inhibition.

METHODS

The effects of the antimetabolites methotrexate and 5-FU were studied in cells from an established human acute myeloblastic leukemia cell line (SK-L7), in murine L1210 leukemia cells either sensitive or resistant to methotrexate, and in cells from malignant effusions in patients with ovarian carcinoma.

Observations of growth inhibition were made in both flask cell culture and in a soft agar clonogenic assay, while the dU suppression test was used to estimate the activity of the de novo pathway to DNA synthesis from dU.

L1210 and SK-L7 cell lines were maintained in vitro by serial passage in flask culture. L1210 and SK-L7 cells were grown in RPMI-1640 medium (Gibco) plus 15% heat-inactivated fetal calf serum (Gibco) and fed at 3- or 4-day intervals by transfer into fresh media at an initial concentration of 1×10^5 cells/ml. Initial at-

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tempts at development of methotrexate-resistant lines from L1210 cells were unsuccessful until cells exposed to methotrexate were allowed to grow, with addition of fresh media until they had reached a final concentration of at least 1×10^6 cells/ml before refeeding and new drug exposure; this required up to 6 weeks' culture. Resistant cell lines were developed as follows: L1210 cells were incubated every other week with a sublethal dose of methotrexate (3.1×10^{-8} M); the cell line derived from this procedure was termed L1210-R2. Additionally, L1210 cells were incubated every other week with a sublethal dose of methotrexate (1.1×10^{-8} M) and the resulting cell line was termed L1210-R3. Both L1210-R2 and L1210-R3 were grown drug-free during alternate weeks.

Methotrexate and 5-FU used in all studies was obtained from commercial sources and diluted in Hanks' tris buffer. Studies comparing the relative sensitivity of the dU suppression test with flask culture and with soft agar culture results using the leukemia cell lines were done concomitantly with the same cell suspension. As seen in figure 1, the L1210 cells were exposed to drug for 24 hours in flask culture, washed free of drug, then divided and grown in both flask culture and soft agar clonogenic culture systems. All studies were performed in triplicate with average results plotted.

Assessment of leukemia cell growth inhibition in flask culture was done as previously described (5). Briefly, log cells at an initial concentration of 1×10^5 cells/ml were incubated with drug for 24 hours at 37°C in a 5% CO_2, 95% air atmosphere. A variety of exposure times were evaluated prior to selecting the 24-hour exposure period. After the 24-hour drug exposure, cells were washed by centrifuging flask contents at 200 $\times$ *g* for 10 minutes at room temperature, washing twice with fresh RPMI-1640 plus 15% fetal calf serum, then resuspending the cell button in 5 ml of fresh media and allowing the cells to grow for 4-7 days. At that time, cell counts were done on a Coulter Model F Counter.

The method of assessment of leukemia cell growth inhibition in the soft agar assay was modified from the

A. L1210 AND SK-L7 LEUKEMIA CELLS
CELLS FROM FLASK CULTURE
VIABILITY CHECK WITH TRYPAN BLUE AND COUNT ON COULTER F
ALIQUOT FOR dU SUPPRESSION TEST
ALIQUOT FOR CELL GROWTH EVALUATION
ADD DRUG TO CULTURE FLASK AND INCUBATE FOR 24 HOURS AT 37°C
CENTRIFUGE AND WASH FREE OF DRUG
ALIQUOT FOR FLASK CULTURE
ALIQUOT FOR SOFT AGAR ASSAY
B. MALIGNANT EFFUSIONS FROM OVARIAN CARCINOMA PATIENTS
LAYER EFFUSION ON FICOLL-HYPAQUE
CENTRIFUGE AT 5°C FOR 20 MINUTES AT 2000 RPM
REMOVE CELLS AT INTERFACE
WASH FREE OF FICOLL-HYPAQUE
CHECK VIABILITY WITH TRYPAN BLUE AND COUNT ON COULTER F
ALIQUOT FOR dU SUPPRESSION TEST
ALIQUOT FOR SOFT AGAR CULTURE
ADD DRUG AND PLATE IMMEDIATELY IN SOFT AGAR CULTURE

FIGURE 1.—Preparation of L1210 and SK-L7 leukemia cells and cells from malignant effusions of patients with ovarian carcinoma for the dU suppression test and cell growth evaluation.

technique of Hamburger and Salmon (6). Single-cell suspensions were incubated with drug in tissue culture flasks for 24 hours at 37°C in a 5% CO_2, 95% air atmosphere. After the 24-hour drug exposure, cells were washed as outlined above and plated in soft agar plates at an initial concentration of 0.5×10^6 cells/ml at 37°C, with 95%-97% humidity, in a 5% CO_2, 95% air atmosphere. Each plate was read and calculated as percent of clones formed in control plates without drug. A variety of criteria have been arbitrarily used to define in vitro drug sensitivity in the soft agar assay (7-9). In this report, plates with < 50% of the control number of clones were termed positive and sensitive to the added drug. Plates were read in 14-28 days.

The dU suppression test was performed in single-cell suspensions in Hanks' tris buffer. The dU suppression test was modified from that described by Herbert and co-workers (1), with the use of a 120-minute incubation with anticancer drugs.

Briefly, single-cell suspensions were incubated with or without cold dU (0.23 ng/ml) and anticancer drug in 0.06 M Hanks' tris buffer, pH 7.4, for 1 hour at 37°C, 5% CO_2, and 95% air. Cell suspensions were then incubated with 1 μg of ^{125}I-iododeoxyuridine (specific activity, 5 mCi/mg) for 60 minutes, washed with cold buffer, precipitated with 10% trichloroacetic acid, and counted on a Beckman Bio gamma spectrometer. The effects of in vitro additions of drugs on dU suppression were always compared to their effects on thymidine incorporation in the absence of dU. The results were expressed in percentages, comparing dU-suppressed ^{125}I-iododeoxyuridine incorporation with the matched nonsuppressed incorporation. In the absence of anticancer drug addition, normally suppressed incorporation was ⩽ 10%. In the presence of anticancer drug, radioactive counts > 10% of control values without added dU were determined to represent an abnormal dU suppression test which indicated a drug-induced block in the de novo pathway (1,2). Results of the dU suppression test were obtained within 4 hours of tumor cell suspension.

The approach used to obtain cells from malignant effusions in patients with ovarian carcinoma for the dU suppression test and clonogenic soft agar assay is outlined in figure 1. The effusions were layered directly on 9% Ficoll and 33.9% Hypaque (1.080 specific gravity) and centrifuged at 5°C for 20 minutes at 500 × *g* (7). The cells at the blind interface were removed and washed free of Ficoll-Hypaque. At this point, viability was checked using trypan blue, and cells were divided for assessment in either the dU suppression test or the soft agar assay. Assessment of ovarian carcinoma effusion cell growth inhibition in the soft agar assay involved continuous drug exposure. Drug was added to single-cell suspensions which were then plated at an initial cell concentration of $0.5\text{-}1.0 \times 10^6$ cells/ml, at 37°C, with 95%-99% humidity, in 5% CO_2 and 95% air.

RESULTS

The sensitive L1210 leukemia cell line demonstrated comparable growth inhibition when exposed to 10^{-7} M methotrexate for 24 hours in flask culture and soft agar systems, with > 50% growth inhibition seen in both (fig 2). The resistant tumor cell lines (L1210-R2 and L1210-R3), which had been growing for some time in the presence of methotrexate, required a one-log higher methotrexate concentration to achieve growth inhibition (table 1).

As seen in table 1, in both sensitive and resistant

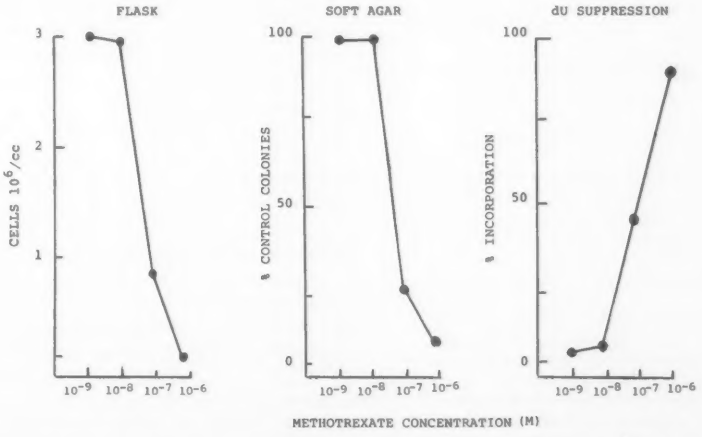

FIGURE 2.—Comparative sensitivity to methotrexate as determined using flask and soft agar culture systems and the dU suppression test in sensitive L1210 cells.

TABLE 1.—Comparison of methotrexate concentration required to achieve growth inhibition (≤ 50% control) in flask and soft agar culture of a positive dU suppression test in murine and human leukemia cell lines

Test	Methotrexate exposure time (hrs)	Methotrexate concentration (M)			
		Sensitive	Resistant		
			R-2	R-3	SK-L7
dU suppression*	2	10^{-7}	10^{-6}	10^{-6}	10^{-7}
Flask culture†	24	10^{-7}	10^{-6}	10^{-6}	10^{-7}
Soft agar culture	24	10^{-7}	10^{-6}	NT‡	NT‡

*dU suppression test was considered positive when radioactive counts were ≥ 10% of controls; it represents a drug-induced block in the de novo pathway to DNA thymidine. Results of the dU suppression test were available in 4 hrs. SD was < 5% in all cases of triplicate assay results. Control cpm in dU suppression tests were 15,400 ± 690 (mean ± SD).

†For flask culture and soft agar culture, > 50% of control growth inhibition was considered a positive result. SD of triplicate assay results was < 5% in all cases. Control colony counts for L1210 cultures were 540 ± 27 colonies/plate and for SK-L7 cultures were 488 ± 33 colonies/plate (mean ± SD).

‡NT = not tested.

L1210 cell lines, methotrexate concentrations resulting in growth inhibition were associated with abnormal dU suppression tests with > 10% incorporation of ^{125}I-iododeoxyuridine. A similar parallel relationship between flask culture growth inhibition and dU suppression test results was seen in SK-L7 human leukemia cells (table 1). The comparability of results obtained using these three methods (flask culture and soft agar culture systems and dU suppression) over a range of methotrexate concentrations (10^{-6} to 10^{-9} M) is illustrated for the sensitive L1210 line in figure 2.

The correlation between dU suppression test results and those achieved using the soft agar clonogenic system with respect to sensitivity to methotrexate and 5-FU for cells from malignant effusions from several patients with ovarian cancer is given in table 2. Generally, excellent correlation was seen; a lack of correlation for sensitivity to methotrexate occurred in one patient

TABLE 2.—Methotrexate and 5-FU concentrations required to achieve growth inhibition in soft agar culture or a positive dU suppression test in human tumor explants from patients with ovarian carcinoma*

Patient No.	Drug	Drug concentration (M)	dU suppression test†		Soft agar†
			Cpm ± SD	% incorporation	% control colonies
1	Methotrexate	Control	14,000 ± 840	6 ± 1	80
		10^{-7}	840 ± 62	19 ± 1‡	76
		10^{-6}	2660 ± 183	45 ± 3‡	64
		10^{-5}	6300 ± 405	64 ± 4‡	48‡
		10^{-4}	8960 ± 434		
2	Methotrexate	Control	15,711 ± 785		
		10^{-7}	89 ± 42	0	100
		10^{-6}	314 ± 89	2 ± 1	100
		10^{-5}	5655 ± 450	36 ± 3‡	38‡
		10^{-4}	6598 ± 389	42 ± 3‡	22‡
3	Methotrexate	Control	16,702 ± 818		
		10^{-7}	835 ± 115	5 ± 1	56
		10^{-6}	1670 ± 272	10 ± 2‡	44‡
		10^{-5}	3507 ± 350	21 ± 2‡	35‡
		10^{-4}	4342 ± 333	26 ± 2‡	22‡
4	5-FU	Control	12,571 ± 490		
		10^{-7}	629 ± 101	5 ± 1	54‡
		10^{-6}	1257 ± 113	10 ± 1‡	46‡
		10^{-5}	1886 ± 189	15 ± 2‡	42‡
		10^{-4}	2514 ± 201	20 ± 2‡	31‡

*Cells were derived from malignant ascites in 3 patients with previously untreated ovarian carcinoma.

†For dU suppression, results are depicted as mean ± SD of triplicate assays. Soft agar clones were also run in triplicate. SD of triplicate colony counts was < 5% in all cases. All control soft agar colony plates contained ≥ 117 colonies/plate (Patient 1, 214 control colonies; Patient 2, 117 control colonies; Patient 3, 151 control colonies; and Patient 4, 411 control colonies).

‡Positive results: for dU suppression, ≥ 10% of control incorporation; for soft agar, ≥ 50% of control colony number inhibition.

at intermediate doses of methotrexate but there was correlation at higher methotrexate concentrations.

DISCUSSION

The clinical utility of the dU suppression test in detecting intracellular folate and vitamin B_{12} deficiency in various clinical megaloblastic states has, in part, been superseded by rapid clinical assay for serum B_{12} and folic acid. Nonetheless, the ability to obtain results within 4 hours using this test (1,2) suggested to us that it could provide useful information on de novo pathway function having relevance to the predictive use of antimetabolites in cancer.

In our studies, the dU suppression test has shown excellent correlation with tumor cell growth in vitro using standard flask culture and soft agar clonogenic assay with regard to inhibitory concentrations of antimetabolites. Major attention has recently focused on the soft agar clonogenic assay as being uniquely useful to predict for clinical anticancer effects of drugs in such tumors as myeloma, melanoma, and ovarian cancer (8-16). However, there are theoretical considerations which limit the clinical application of the soft agar clonogenic assay in certain situations. For example, in adjuvant therapy regimens, increasing emphasis is being placed on perioperative or preoperative therapy (17) with shorter, more intensive treatment courses (18). Using the soft agar methodology, results may not provide information rapidly enough to direct such regimens. For antimetabolites acting on the de novo pathway to DNA synthesis, the dU suppression test offers the potential for rapid assessment of sensitivity since results are obtainable within 4 hours of sample collection. In diseases such as breast and oropharyngeal carcinoma, same-day information about sensitivity to methotrexate and 5-FU could facilitate prospective trials on the efficacy of in vitro testing. A further advantage of the dU suppression test is that the test does not depend on selection and subsequent growth of a subset of clonogenic cells. Consequently, once an adequate amount of tumor is available, a result can always be obtained. It should be emphasized that the period of exposure to methotrexate was much shorter in the dU suppression test than that utilized in the flask culture and soft agar assays. Therefore, the lack of correlation seen in one clinical specimen at lower methotrexate dosage levels suggests that further pilot human tumor studies may be required to define the optimal period of antimetabolite exposure in the dU suppression test assay before large-scale prospective clinical assessment of the predictive capacity of this test is undertaken.

At present, a number of systems have been proposed as being predictive of anticancer drug sensitivity (11,12,19-21), and clinical testing will be required to evaluate the comparative efficacy of these in vitro systems. It may well be that several different in vitro testing procedures may be required to optimize predictive ability.

In the present report, we evaluated not only the dU suppression test, but also compared two tests of tumor growth inhibition. It is noteworthy that, following 24 hours of exposure to methotrexate, a similar inhibitory concentration was identified using either a flask culture system or soft agar clonogenic assay system. To know whether direct correlation of results obtained using these two culture systems also occurs for other cytotoxic agents with different mechanisms of action must await further testing.

Our studies with methotrexate-sensitive and -resistant cell lines indicate that the dU suppression test will, in fact, reflect important differences in cell sensitivity to antimetabolites. The biochemical features of our methotrexate-resistant cell lines have not been characterized but could reflect either drug transport or dihydrofolate reductase enzyme induction changes. Pragmatically, the mechanism of such resistance may not be relevant to the observations in this study, since drug cytotoxicity for methotrexate is likely to be ultimately mediated via thymidylate synthetase inhibition. In this report, only the antimetabolites methotrexate and 5-FU have been evaluated. Marrow megaloblastosis, however, is a common finding in patients treated with a variety of anticancer drugs. Therefore, additional studies are needed to delineate which other drugs may influence the dU suppression test and offer the potential for clinical predictive testing.

In summary, the dU suppression test offers the potential for simple, rapid assessment of tumor sensitivity to antimetabolite therapy. Prospective correlation is needed to evaluate the test's potential to predict response of patients to antimetabolites.

REFERENCES

1. HERBERT V, TISMAN G, GO LT, ET AL. The dU suppression test using ^{125}I-UdR to define biochemical megaloblastosis. Br J Haematol 24:713-723, 1973.
2. COLMAN N, and HERBERT V. Abnormal lymphocyte deoxyuridine suppression test: a reliable indicator of decreased lymphocyte folate levels. Am J Hematol 8:169-174, 1980.
3. WEKHEISER WC. Specific binding of 4-amino folic acid analogues by folic acid reductase. J Biol Chem 236:888-893, 1961.
4. HEIDELBERGER C. Fluorinated pyrimidines and their nucleosides, antineoplastic and immunosuppressive agents. *In* Handbook of Experimental Pharmacology. New York, Springer-Verlag, 1974, pp 193-221.
5. BLOCK JB, ISACOFF W, and DIETRICH M. Amphotericin B effects in tissue culture cell lines. Cancer Treat Rep 63:643-645, 1979.
6. HAMBURGER AW, and SALMON SE. Primary bioassay of human tumor stem cells. Science 197:461-463, 1977.
7. JOHNSON PA, and ROSSOF AH. The role of the human tumor stem cell assay in medical oncology. Arch Intern Med 143:111-114, 1983.
8. SELBY P, BUICK RN, and TANNOCK I. A critical appraisal of the "human stem cell assay." N Engl J Med 308:129-134, 1983.

9. MEYSKENS FL, MOON TE, DANA B, ET AL. Quantitation of drug sensitivity by human metastatic melanoma colony forming units. Br J Cancer 44:787-797 1981.
10. LIVINGSTON RB, AMBRIS U, GEORGE SL, ET AL. In vitro determination of thymidine-3H labeling index in human solid tumors. Cancer Res 34:1376-1380, 1974.
11. SALMON SE, HAMBURGER AW, SOCHLEN B, ET AL. Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. N Engl J Med 298:1321-1327, 1978.
12. VON HOFF DD, CASPER J, BRADLEY E, ET AL. Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 70: 1027-1032, 1981.
13. VON HOFF DD, PAGE C, HARRIS G, ET AL. Prospective clinic trial of a human tumor cloning system. Proc Am Assoc Cancer Res and ASCO 22:156, 1981.
14. MANN ED, STORM FK, KERN DH, ET AL. Predictive value of the clonogenic assay in the treatment of melanoma with DTIC and DTIC + hyperthermia. Proc Am Assoc Cancer Res and ASCO 22:155, 1981.
15. TISMAN G, and WU SJG. The clinical and laboratory evaluation of a modified tumor cell assay for the in vitro determination of human tumor cell drug sensitivity. Proc Am Assoc Cancer Res and ASCO 22:364, 1981.
16. ALBERTS DS, CHEN HSG, YOUNG AL, ET AL. Improved survival for relapsing ovarian cancer patients using the human tumor stem cell assay to select chemotherapy. Proc Am Assoc Cancer Res and ASCO 22:462, 1981.
17. PRICE LA, and HILL BT. Improved survival in patients responding to initial chemotherapy prior to local therapy in squamous cell carcinoma of the head and neck. Proc Am Assoc Cancer Res and ASCO 22:535, 1981.
18. NISSEN-MEYER R, KJELLEGREN K, MALMIA K, ET AL. Surgical adjuvant chemotherapy results with one short course with cyclophosphamide after mastectomy for breast cancer. Cancer 41: 2088-2098, 1978.
19. TISMAN G, HERBERT V, and EDLIS H. Determination of therapeutic index of drugs by in vitro sensitivity tests using human host and tumor cell suspensions. Cancer Chemother Rep 57:11-19, 1973.
20. DIADONE ME. Clinical relevance of an in vitro antimetabolite assay for monitoring human tumor chemosensitivity. *In* Adjuvant Treatment of Cancer III. New York, Grune and Stratton, Inc, 1981.
21. WEISENTHAL LM, and MARSDEN J. A novel dye exclusion assay for predicting response to cancer chemotherapy. Proc Am Assoc Cancer Res and ASCO 22:155, 1981.

Differences in Lipid Composition of Doxorubicin-Sensitive and -Resistant P388 Cells[1]

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An anthracycline-resistant subline of P388 murine leukemia cells, P388/ADR, has been shown to have an altered total lipid content and composition when compared to the parent line. When specific lipids of the cell lines are compared, it is found that P388/ADR cells contain approximately 3.6 times the amount of triglycerides as P388 cells. Although no differences are noted in the amounts of unesterified cholesterol or total phospholipids in the two cell lines, they do differ in specific phospholipid patterns. P388/ADR cells contain relatively less phosphatidylcholine and more sphingomyelin than drug-sensitive cells. No differences are observed in the content of phosphatidylethanolamine and cardiolipin. The difference in phosphatidylcholine/sphingomyelin ratio (5.57 for ADR-sensitive cells and 3.28 for ADR-resistant cells) may account for the previously observed difference in plasma membrane lipid structural order between these cell lines. Measurements of the relative activity of phosphocholine transferase in the two lines, using the rate of incorporation of ^{3}H-choline into phosphatidylcholine, indicate that lower phosphocholine transferase activity in P388/ADR cells may account for the observed changes in cellular lipid and phospholipid composition. [Cancer Treat Rep 68:637–641, 1984]

Resistance to doxorubicin (ADR) in P388 murine leukemia cells is associated with cross-resistance to other anthracyclines as well as to many nonanthracycline cytotoxic drugs; at least part of the relative resistance of these cells is related to decreased intracellular drug accumulation compared to drug-sensitive cells (*see* references listed in ref 1). Recently, we have shown that the degree of structural order of plasma membrane lipids is higher in ADR-resistant P388 cells (P388/ADR) and have suggested that these differences may relate to the lower intracellular accumulation of drugs (1). It was suggested that these differences between ADR-sensitive and -resistant P388 cells may reflect differences in the lipid composition of plasma membranes of these cell lines. Further, measurements of binding of 1,6-diphenylhexatriene (DPH) and fluorescence polarization measurements revealed that these cell lines have differing amounts of some intracellular lipids. Staining the cells with Oil Red O and cell fractionation have also indicated that P388/ADR cells contain more cytoplasmic lipids than drug-sensitive cells. These cellular lipids were shown to have a very low steady-state DPH fluorescence polarization (1).

The purpose of the present study was to characterize the intracellular lipid composition of ADR-sensitive and -resistant P388 cells and to examine the phospholipid composition of the two cell lines as an indication of plasma membrane differences. The differences observed correlate with the functional observations outlined above.

METHODS

Cell culture.—ADR-sensitive and -resistant P388 cells were grown in RPMI-1640 medium (Gibco, Grand Island, NY) and supplemented with 10% heat-inactivated fetal calf serum (Gibco), 10 μM 2-mercaptoethanol (Sigma Chemical Co, St. Louis, MO), 50 units/ml of penicillin base, and 50 μg/ml of streptomycin base (both from Gibco). Cell densities were measured using a Coulter counter Model ZB_1 (Coulter Electronics). Cells were transferred to fresh medium every 4 days. Initial cell densities were 10^5 cells/ml and after 4 days were 1–2 × 10^6

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cells/ml. Every five to ten transfers, the effect of 1×10^{-7} M ADR on the growth rate of both cell lines was measured to verify the constancy of their differential sensitivity to ADR. All assays were performed with cells harvested on the fourth day of growth.

Lipid extraction.—Cells (10^8) were pelleted at 700 × *g* for 10 minutes and washed twice with 45 ml of phosphate-buffered saline (PBS). The washed cells were resuspended in 2 ml of water, and 8 ml of methanol:chloroform (2:1, vol/vol) was added. The lipids were extracted using the method of Bligh and Dyer (2). Following phase separation, the chloroform phase was evaporated under a stream of N_2. The lipids were redissolved in 0.2 ml of chloroform, deaerated with N_2, and stored at -20°C until assayed.

Neutral lipids separation and quantitation.—The lipids extracted from the ADR-sensitive and -resistant P388 cells were separated by thin-layer chromatography on silica gel G plates (E. Merck, W Germany). Five micrograms of each standard lipid (Supelco, Inc) was also spotted on the same plates. Petroleum ether (boiling point, 60°C-70°C):diethyl ether:acetic acid 90:10:1 (vol/vol) was employed as a solvent (3). After developing, the plates were dried at room temperature and sprayed with a 3% cupric acetate solution in 8% aqueous H_3PO_4 (4). The plates were heated to 200°C for 10 minutes. Optical densities of the charred spots were monitored at 480 nm using a Zeiss M4 QIII chromatogram spectrometer. Free cholesterol was determined by the enzymatic fluorometric method of Heider and Boyett (5). The whole-cell content of triglycerides was determined using a Gilford diagnostic triglyceride (color) reagent (Gilford Instrument Laboratories Inc, Cleveland, OH).

Analyses of phospholipids.—Inorganic phosphate was released from phospholipids by the method of Dittmer and Wells (6). Aliquots of chloroform extracts of both cell lines were transferred to 12 × 75-mm glass tubes and the chloroform was evaporated to dryness; 50 µl of 70% perchloric acid was then added and the tubes were heated to 200°C in a sandbath for 30 minutes or until the sample cleared. Inorganic phosphate was determined by the molybdate-malachite green method of Itaya and Ui (7). Phospholipids were separated by thin-layer chromatography on silica gel H plates developed in a mixture of chloroform:methanol:water:acetic acid 65:43:3:1 (by vol) (8). Standard reference phospholipids were obtained from Supelco, Inc. Spots were visualized and quantitated as described above.

Incorporation of choline and methionine into cell phospholipids.—Washed cells (2×10^7) were incubated in 10 ml of RPMI-1640 medium with 50 µCi of [methyl-^{3}H]-choline chloride (specific activity, 80 Ci/mmol, New England Nuclear, Boston, MA) at 37°C for variable periods of time. At the end of the incubation period, the tubes were placed on ice and centrifuged at 700 × *g* for 10 minutes, and the supernatant was aspirated and discarded. The pellets were washed twice with 10 ml of ice-cold PBS and then resuspended in 2 ml of distilled water. Lipids were extracted and phospholipids chromatographed as described above. The only change in these procedures was the placement of an additional 10 µl of a mixture containing sphingomyelin, phosphatidylcholine, and phosphoethandamine (0.1% of each phospholipid) on each of the thin-layer chromatographic sample spots prior to development of the plates, to enhance the migration of the labeled compounds. After visualization with iodine vapor, the phospholipid spots were identified, marked, and then scraped into scintillation vials. Exactly the same experimental procedure was performed with freshly obtained (within 2 weeks) [methyl-^{3}H]-L-methionine (specific activity, 12 Ci/mmol, New England Nuclear).

RESULTS

As shown in table 1, there was no difference in free cholesterol content between drug-sensitive and drug-resistant cells. The same result was obtained when free cholesterol was measured by the cholesterol oxidase method (5). The major difference in neutral lipids between the cell lines was in the content of triglycerides. As shown in table 1, the content of triglycerides in P388/ADR cells was more than three and one-half times higher than in P388 cells. This difference in triglyceride content was also verified by the Gilford diagnostic method for triglycerides. Cholesterol esters and another unidentified neutral lipid (R_f 0.381) were present only in trace amounts.

Although there was no significant difference in whole-cell lipid phosphorus between drug-sensitive and -resistant P388 cells, separating the phospholipids on thin-layer chromatography revealed differences in their relative quantities (table 2). The major phospholipids found were phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and cardiolipin. P388/ADR cells contained more sphingomyelin and less phosphatidylcholine compared to drug-sensitive cells, while there were no signifi-

TABLE 1.—Distribution of neutral lipids in ADR-sensitive and -resistant P388 cells

	µg/10^6 cells, mean ± SD (n = 4)		
	P388	P388/ADR	P388 / P388/ADR
Free cholesterol	0.84 ± 0.02	0.83 ± 0.08	1.01
Triglycerides	0.58 ± 0.06	2.08 ± 0.17	0.28

TABLE 2.—Distribution of phospholipids in ADR-sensitive and -resistant P388 cells

	$\mu g/10^6$ cells, mean ± SD (n = 4)		
	P388	P388/ADR	P388 P388/ADR
Sphingomyelin	0.37 ± 0.04	0.54 ± 0.04	0.69
Phosphatidylcholine	2.06 ± 0.15	1.77 ± 0.12	1.16
Phosphatidylethanolamine	0.63 ± 0.04	0.65 ± 0.11	0.97
Cardiolipin	0.35 ± 0.03	0.32 ± 0.01	1.09

cant differences in the content of phosphatidylethanolamine and cardiolipin. The phosphatidylcholine/sphingomyelin ratio in P388/ADR cells was only 59% of the ratio in drug-sensitive cells. Phosphatidylserine and phosphoinositol were measured in only trace amounts in both lines.

Incorporation of ^{3}H-choline by cells of both lines was also compared. In P388 cells after 90 minutes of incubation with ^{3}H-choline, the fraction of radioactivity bound to cells and not extracted into the chloroform phase was fivefold higher than that found in the chloroform phase. After lipid extraction there was no difference between the cell lines in the radioactivity remaining in the water phase. However, the radioactivity extracted by chloroform from P388 cells was 50% higher than that found in chloroform extracts of P388/ADR cells. As shown in table 3, this difference was also found in the ^{3}H-choline counts incorporated into the phosphatidylcholine fraction of these cells. Less than 2% of the chloroform radioactivity remained at the starting point or at the sphingomyelin and phosphatidylethanolamine spots. The counts in these spots were not significantly different between these cell lines. Compared to choline, the amount of ^{3}H-methionine bound to the cells was low. As shown in table 4, the amount of lipid-associated radioactivity was higher in ADR-resistant cells compared to drug-sensitive cells. However, the counts in the discrete phospholipid spots on the chromatograph were insufficient to determine their relative degree of labeling by ^{3}H-methionine.

DISCUSSION

The most remarkable difference in the lipid composition between ADR-sensitive and -resistant cells was found in the triglyceride content (table 1). Triglycerides have a very low steady-state DPH fluorescence polarization (9), close to the fluorescence polarization we obtained for the isolated intracellular lipid fraction (1). Furthermore, since triglycerides are not present in plasma membranes (9–11), it is clear that the previously reported difference in intracellular lipid content between these cell lines (1) is primarily a reflection of the much higher content of triglycerides in ADR-resistant cells.

TABLE 3.—Incorporation of ^{3}H-choline into phosphatidylcholine of ADR-sensitive and -resistant P388 cells

	Incubation time (mins)	Cpm in phosphatidylcholine extracted from 2×10^7 cells, mean ± SD (n = 4)
P388	30	4200 ± 11
	90	17,240 ± 2670
P388/ADR	30	2808 ± 238
	90	11,444 ± 2721

The effects of unesterified cholesterol and phospholipid content and the cholesterol/phospholipid ratio on membrane lipid structural order are well-known (12,13). However, no significant differences in the content of free cholesterol or total phospholipid were found between ADR-sensitive and -resistant P388 cells. There is increasing evidence that membrane lipid structural order may also be affected by the composition of phospholipids in the membrane (10,12–15). Among the major phospholipids found in these cells, there was no difference in the content of phosphatidylethanolamine and cardiolipin; however, the phosphatidylcholine/sphingomyelin ratio of P388/ADR cells was significantly lower than that of ADR-sensitive cells. Gilmore et al (14) have shown that changes induced in cell phospholipid composition were reflected in membranes (plasma, microsomal, and mitochondrial). Therefore, it is likely that the difference found between ADR-sensitive and -resistant cells in the ratio of the whole-cell phosphatidylcholine/sphingomyelin is also reflecting differences in phospholipid ratios within plasma membranes of the cells. Based on studies with liposomes, it has been shown that the phosphatidylcholine/sphingomyelin ratio is inversely related to the degree of lipid structural order (16,17). Thus, it seems reasonable to assume that the lower ratio of these phospholipids in P388/ADR cells is associated with higher plasma membrane lipid structural order, as previously reported (1), leading to reduced drug accumulation.

The differences in triglyceride content, phosphatidylcholine content, and sphingomyelin content may occur independently when P388 cells acquire resistance to the drug. Another plausible explanation is that they result from a single heritable difference between these cell lines. Considering the major synthetic pathways of these lipids (fig 1), the most probable suggestion that emerges is that P388/ADR cells have a lower activity of phosphocholine transferase than P388 cells. This results not only in reduced production of phosphatidylcholine but also, as

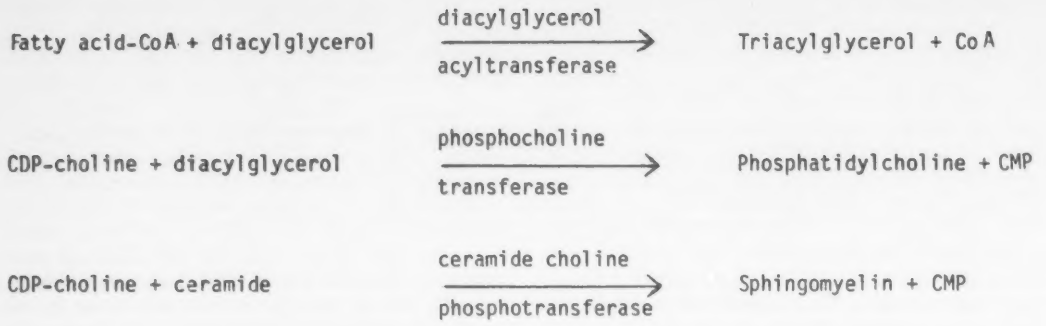

FIGURE 1.—Major synthetic pathways of triacylglycerol, phosphatidylcholine, and sphingomyelin.

a result of substrate accumulation, in increased synthesis of triglycerides and sphingomyelin. This hypothesis was partially tested in experiments where the incorporation of ^{3}H-choline into cell lipids was studied (table 3). Production of labeled phosphatidylcholine was significantly lower in drug-resistant cells. The non-lipid-associated radioactivity accumulated by the cells was much higher than that incorporated into lipids and was equal in both cell lines. Therefore, it is unlikely that choline uptake is the limiting step in the production of labeled phosphatidylcholine. In addition to the major synthetic pathway (18), the contribution of the methylation pathway to the production of phosphatidylcholine (19,20) and sphingomyelin (21) was also studied. As shown in table 4, the incorporation of radioactivity originating from [^{3}H-methyl]-L-methionine into lipids was negligible compared to that of radioactivity originating from ^{3}H-choline (table 3). Kannagi et al (22) have shown that production of phosphatidylcholine by *N*-methylation results in considerably higher arachidonyl phosphatidylcholine than that occurring through choline incorporation. Since this reaction is associated with prostaglandin production, it is important to note that although the incorporation of methionine radioactivity into cell lipid was much lower than ^{3}H-choline, it was significantly higher in P388/ADR cells than in drug-sensitive cells. We have previously noted that production of prostaglandins is higher in drug-resistant cells than in drug-sensitive cells. It remains to be seen whether the differences in lipid composition reported here between ADR-sensitive and -resistant P388 cells are characteristic only of these cell lines or whether they are associated with acquired ADR resistance in other tumors as well.

TABLE 4.—^{3}H-methionine incorporation into lipids of ADR-sensitive and -resistant P388 cells

	Incubation time (mins)	Cpm in lipids extracted from 1.5 × 10^{7} cells, mean ± SD (n = 4)
P388	30	95 ± 4
	120	587 ± 38
P388/ADR	30	228 ± 29
	120	723 ± 63

REFERENCES

1. RAMU A, GLAUBIGER D, MAGRATH IT, ET AL. Plasma membrane lipid structural order in doxorubicin sensitive and resistant P388 cells. Cancer Res 43:5533-5537, 1983.
2. BLIGH EG, and DYER WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917, 1959.
3. MANGOLD HK, and MALINS DC. Fractionation of fats, oils and waxes on thin layers of silicic acid. J Am Oil Chem Soc 37:383-385, 1960.
4. FEWSTER ME, BURNS BJ, and MEAD JF. Quantitative densitometric thin-layer chromatography of lipids using copper acetate reagent. J Chromatogr 43:120-126, 1969.
5. HEIDER JG, and BOYETT RL. The picomole determination of free and total cholesterol in cells in culture. J Lipid Res 19:514-518, 1978.
6. DITTMER JC, and WELLS MA. Quantitative and qualitative analysis of lipids and lipid components. Methods Enzymol 14:482-530, 1969.
7. ITAYA K, and UI M. A new micromethod for the colorimetric determination of inorganic phosphate. Clin Chim Acta 14:361-366, 1966.
8. OWENS K. A two-dimensional thin-layer chromatographic procedure for the estimation of plasmalogens. Biochem J 100:354-361, 1966.
9. VAN HOEVEN RP, VAN BLITTERSWIJK WJ, and EMMELOT P. Fluorescence polarization measurements on normal and tumor cells and their corresponding plasma membranes. Biochim Biophys Acta 551:44-54, 1979.
10. ESKO JD, GILMORE JR, and GLASER M. Use of a fluorescent probe to determine the viscosity of LM cell membranes with altered phospholipid compositions. Biochemistry 16:1881-1890, 1977.
11. STUBBS CD, TSANG WM, BELIN J, ET AL. Incubation of exogenous fatty acids with lymphocytes, changes in fatty acid composition and effects on the rotational relaxation time of 1,6-diphenyl-1,3,5-hexatriene. Biochemistry 19:2756-2762, 1980.
12. SHINITZKY M, and BARENHOLZ Y. Fluidity parameters of lipid regions determined by fluorescence polarization. Biochim Biophys Acta 515:367-394, 1978.
13. SHINITZKY M, and HENKART SP. Fluidity of cell membranes—current concepts and trends. Int Rev Cytol 60:121-147, 1979.
14. GILMORE R, COHN N, and GLASER M. Fluidity of LM cell membranes with modified lipid compositions as determined with 1,6-diphenyl-1,3,5 hexatriene. Biochemistry 18:1042-1049, 1979.
15. GILMORE R, COHN N, and GLASER M. Rotational relaxation times of 1,6-diphenyl-1,3,5-hexatriene in phospholipids isolated from LM cell membranes. Effects of phospholipid polar head-group and fatty acid composition. Biochemistry 18:1050-1056, 1979.
16. SHINITZKY M, and BARENHOLZ Y. Dynamics of the hydrocarbon lay-

er in liposomes of lecithin and sphingomyelin containing dicetylphosphate. J Biol Chem 249:2652–2657, 1974.
17. SCHMIDT CF, BARENHOLZ Y, and THOMPSON TE. A nuclear magnetic resonance study of sphingomyelin in bilayer systems. Biochemistry 16:2649–2656, 1977.
18. KENNEDY EP, and WEISS SB. The function of cytidine coenzymes in the biosynthesis of phospholipids. J Biol Chem 222:193–214, 1956.
19. BREMER J, and GREENBERG DM. Methyl transferring enzyme system of microsomes in the biosynthesis of lecithin (phosphatidylcholine). Biochim Biophys Acta 46:205–216, 1961.
20. HIRATA J, CORCORAN BA, VENKAT K, ET AL. Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. Proc Natl Acad Sci USA 76:2640–2643, 1979.
21. SINCLAIR AJ. The incorporation of ethanolamine and methionine into sphingomyelin in Ehrlich ascites tumor cells. Can J Biochem 49:700–708, 1971.
22. KANNAGI R, KOIZUMI K, HATA-TANOUE S, ET AL. Mobilization of arachidonic acid from phosphatidylethanolamine fraction to phosphatidylcholine fraction in platelets. Biochem Biophys Res Commun 96:711–718, 1980.

Chemotherapy-Increased Antineoplastic Effects of Antibody-Toxin Conjugates[1,2]

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A model, employing murine L1210 leukemia to which the artificial determinant TNP was bound in vitro and an anti-DNP antibody–ricin A-chain conjugate, was used to explore the in vivo therapeutic potential of combined immunotoxin-chemotherapy treatment. Under conditions in which immunotoxin and chemotherapy as single therapies had only marginal therapeutic activity, their combined use was markedly effective. [Cancer Treat Rep 68:643–645, 1984]

With the aim of selectively delivering toxic agents to tumor cells, a good deal of interest has centered over the past two decades on the use of immunotoxins, ie, conjugates between antibodies to surface determinants present on cancer cells and cytotoxic drugs or natural toxins, such as diphtheria toxin or ricin A-chain (1,2). Interest in this approach has recently become more pronounced with the availability of tumor-directed monoclonal antibodies, which, because of their greater specificity, can be expected to confer greater efficacy to these hybrid molecules.

In vivo data on the antineoplastic effects of different immunotoxins have been obtained in experimental tumors (2–6). Results are clearly remarkable. However, it is difficult to believe that in the very near future immunotoxins will be used alone in human tumor therapy. It seems more likely that immunotoxins combined with other forms of control of tumor growth will be used. At present, a lack of direct information on the efficacy of this combination approach still exists.

To initially explore the therapeutic potential of immunotoxin treatment in conjunction with chemotherapy, an anti-DNP–ricin A-chain complex prepared with conventional goat antibody was used in an experimental system consisting of L1210 leukemia to which the cross-reacting hapten TNP was bound in vitro prior to its transplant into syngeneic hosts. The effects of immunotoxin-chemotherapy combined treatments will be presented here.

MATERIALS AND METHODS

DBA/2 and CDF_1 female mice (8–10 weeks of age at the start of experiments, obtained from Charles River, Italy) were used for the maintenance (by weekly ip transplants) of L1210 leukemia and for the in vivo tests. The preparation of ricin A-chain, a polyclonal goat Ig anti-DNP antiserum, and the anti-DNP–immunotoxin conjugate (DS9) containing A-chain and antibody molecules in a 1:1 molar ratio has been described in detail previously (7).

The dose of DS9 employed is expressed in terms of the quantity of A-chain present in the conjugate. Labeling of L1210 leukemia cells with TNP (L1210-TNP) or with phosphate-buffered saline (L1210-PBS) was performed at 4°C following the conditions described elsewhere (7). Ricin A-chain, anti-DNP antibody, or DS9 was given ip on Day 0, 1 hour after tumor transplant. Doxorubicin (DXR; Farmitalia, Milan, Italy) and cyclophosphamide (CY; Asta Werke, Bielefeld, W Germany) were dissolved in sterile saline and given iv or ip, respectively, on Day 1. Statistical analyses were performed with the Mann-Whitney test for survival times and with the Fisher exact test for long-term survivors (LTS).

RESULTS

Under the conditions employed, the in vitro labeling of L1210 cells with TNP did not significantly affect their subsequent in vivo growth as revealed by superimposable

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TABLE 1.—Effect of DS9 on L1210 tumor growth*

Tumor inoculum	Treatment†	MST in days (range)	LTS‡ (No. of survivors/No. of mice)
10^2			
L1210-PBS	PBS	11.5 (11-12)	0/6
L1210-PBS	DS9	11.5 (11-13)	0/6
L1210-TNP	PBS	12.5 (12-14)	0/6
L1210-TNP	DS9	14.5 (13-17)§	1/6
10^4			
L1210-PBS	PBS	9.5 (8-10)	0/6
L1210-PBS	DS9	10.0 (9-10)	0/6
L1210-TNP	PBS	10.5 (10-11)	0/6
L1210-TNP	DS9	12.5 (12-13)§	0/6
10^3			
L1210-PBS	PBS	12.0 (11-12)	0/10
L1210-PBS	DS9	12.0 (11-13)	0/10
L1210-TNP	PBS	11.5 (11-12)	0/10
L1210-TNP	DS9	15.5 (14-16)§	4/10§
L1210-TNP	Ab	11.5 (11-14)	0/10
L1210-TNP	ChA	14.0 (12-17)§,¶	0/10
L1210-TNP	Ab + ChA	14.0 (13-16)§,¶	2/10

*L1210 tumor cells were incubated with PBS (L1210-PBS) or TNBS for TNP labeling (L1210-TNP).

†Ab = anti-DNP antibodies, 250 μg/mouse ip, equivalent to amount present in DS9; ChA = ricin A-chain, 50 μg/mouse ip. PBS, DS9, Ab, or ChA was given ip 1 hr after tumor transplantation.

‡Survival > 90 days.

§$P < 0.05$ relative to PBS treatment.

¶$P < 0.05$ relative to DS9 treatment.

median survival times (MST) in mice transplanted with L1210-TNP and control L1210-PBS cells (table 1). In animals transplanted with 10^2, 10^3, or 10^4 L1210-TNP cells, single ip injections of DS9, containing the equivalent of 50 μg/mouse of ricin A-chain, were capable of significantly prolonging MST, while the same DS9 treatment had no antitumor effect in L1210-PBS-grafted mice. The antineoplastic activity of DS9 in mice transplanted with 10^3 L1210-TNP cells was greater than that seen with the corresponding doses of anti-DNP antiserum (250 μg) or ricin A-chain alone or of both materials given simultaneously in noncomplexed form. In fact, although significant therapeutic activity under these conditions was seen with ricin A-chain alone or in combination with anti-DNP antiserum, the effect observed with either treatment was significantly and consistently inferior to that of DS9 as judged on the basis of percent increase in MST and/or proportion of LTS. Anti-DNP antiserum alone did not influence MST. LTS (survival > 90 days) after DS9 were seen, however, in only two (one of ten and four of ten animals) of the four experiments performed with 10^3 L1210-TNP cells.

The possibility of increasing DS9 effectiveness by its combination with chemotherapy was next tested in mice transplanted with 10^3 L1210-TNP and given DXR or injected with 10^4 L1210-TNP and treated with CY. As shown in table 2, the therapeutic effectiveness of the combined treatment of DS9 and chemotherapy was clearly superior to that seen with either treatment alone. This superiority is revealed by the finding that DS9 treatment is associated not only with a further prolongation in MST over that seen with chemotherapy alone, but especially with significant increases in the percentage of LTS. Employing the highest doses of DXR and CY, LTS rates of 60% and 80%, respectively, were seen after combination of the drug with DS9.

CONCLUSIONS

The objective of this study was limited to obtaining initial information in experimental conditions on the possibility of effectively combining antibody-toxin conjugates and conventional cancer chemotherapy. We have shown that hybrid molecules containing ricin A-chain and antibodies against an antigen present on tumor cells (TNP artificial antigen) not only can be effective in the control of L1210 leukemia growth but also can increase the antitumor effects of two widely used chemotherapeutic drugs, DXR and CY. The immunotoxin, given alone, is able to increase survival and, occasionally, to induce "cures" (> 90 days) in mice transplanted with up to 10^4 cells of a very fast growing tumor (L1210), which kills, in 20 days, 100% of mice inoculated with less than ten cells (8). On the other hand, immunotoxin treatment markedly increased the antitumor efficacy of the subsequent chemotherapy performed with drugs, such as CY and DXR, which are clinically widely employed. Under conditions in which the conjugate alone had limited therapeutic efficacy, this advantage was seen not only with drug doses already effective per se but also with

TABLE 2.—Effect of DS9-chemotherapy combination on L1210 tumor growth*

Tumor inoculum	Treatment†	MST in days (range)	LTS (No. of survivors/No. of mice)
10^3			
L1210-TNP	PBS	11.5 (11-12)	0/10
L1210-TNP	DS9	15.0 (13-21)	0/10
L1210-TNP	DXR, 2.5 mg/kg	12.0 (11-16)	0/10
L1210-TNP	DXR, 5.0 mg/kg	16.0 (13-19)	0/10
L1210-TNP	DXR, 7.5 mg/kg	16.0 (13-18)	2/10
L1210-TNP	DXR, 2.5 mg/kg + DS9	19.0 (15-22)‡	3/10
L1210-TNP	DXR, 5.0 mg/kg + DS9	17.5 (14-22)‡	3/10
L1210-TNP	DXR, 7.5 mg/kg + DS9	> 90.0 (9 to > 90)‡	6/10§
10^4			
L1210-TNP	PBS	10.0 (9-12)	0/10
L1210-TNP	DS9	13.5 (12-14)	1/10
L1210-TNP	CY, 25 mg/kg	13.0 (10-18)	0/10
L1210-TNP	CY, 50 mg/kg	14.5 (12-17)	2/10
L1210-TNP	CY, 75 mg/kg	15.5 (13-24)	0/10
L1210-TNP	CY, 25 mg/kg + DS9	15.0 (13-17)‡	2/10
L1210-TNP	CY, 50 mg/kg + DS9	20.0 (14 to > 90)‡	5/10
L1210-TNP	CY, 75 mg/kg + DS9	> 90.0 (10 to > 90)‡	8/10‡

*See explanations in table 1.
†DXR or CY given iv or ip, respectively, on Day 1.
‡$P < 0.05$ relative to drug alone.
§$P < 0.1$ relative to drug alone.

marginally effective and ineffective doses (2.5 mg/kg of DXR and 25 mg/kg of CY).

This type of finding may have direct implications for the clinical use of immunotoxins, not only because of the possibility of increasing the current therapy but also because of the possibility of reducing chemotherapy-associated toxicity without therapeutic loss.

Although a larger series of experimental tumors must be investigated under less artificial conditions than were used here before the true therapeutic potential of this combined treatment can be properly assessed, results obtained appear to justify interest in the further testing of this approach. Furthermore, no attempts were made here to determine the optimal sequence of the two treatments.

REFERENCES

1. THORPE PE, ROSS WCJ, CUMBER AJ, ET AL. Toxicity of diphtheria toxin for lymphoblastoid cells is increased by conjugation to antilymphocytic globulin. Nature 271:752-755, 1978.
2. MOOLTEN FL, CAPPAREL NJ, ZAJDEL SH, ET AL. Antitumor effects of antibody-diphtheria toxin conjugates. II. Immunotherapy with conjugates directed against tumor antigens induced by Simian virus 40. JNCI 55:473-477, 1975.
3. BLYTHMAN HE, CASELLAS P, GROS O, ET AL. Immunotoxins: hybrid molecules of monoclonal antibodies and a toxin subunit specifically kill tumour cells. Nature 290:145-146, 1981.
4. TROWBRIDGE IS, and DOMINGO DL. Anti-transferrin receptor monoclonal antibody and toxin-antibody conjugates affect growth of human tumour cells. Nature 294:171-173, 1981.
5. VITETTA ES, KROLICK KA, and UHR JW. Neoplastic B cells as targets for antibody-ricin A chain immunotoxins. Immunol Rev 62:159-183, 1982.
6. SETO M, UMEMOTO N, SAITO M, ET AL. Monoclonal anti-MM46 antibody: ricin A chain conjugate: in vitro and in vivo antitumor activity. Cancer Res 42:5209-5215, 1982.
7. JANSEN FK, BLYTHMAN HE, CARRIERE D, ET AL. High specific cytotoxicity of antibody-toxin hybrid molecules (immunotoxins) for target cells. Immunol Lett 2:97-102, 1980.
8. SKIPPER HE, SCHABEL FM, JR, and WILCOX WS. Experimental evaluation of potential anticancer agents. XII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep 35:1-111, 1964.

Treatment of Metastatic Colorectal Carcinoma With 5-FU, Mitomycin, Vincristine, and Methotrexate[1,2]

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Fifty-three patients with advanced metastatic colorectal carcinoma were treated every 4–5 weeks with a chemotherapeutic regimen consisting of 5-FU (300 mg/m² iv on Days 1–5), mitomycin (10 mg/m² iv on Day 1 only [repeated every other course]), vincristine (0.75 mg/m² on Days 1 and 5), and methotrexate (8.0 mg/m² iv on Days 1 and 5). The overall response rate was 43.4%, with three patients (5.7%) achieving complete response (mean duration, 13.3 months) and 20 patients (37.7%) achieving partial response (mean duration, 6.3 months). Stable disease was not considered to be a response category in this report. The mean overall response duration was 7.8 months. The observed mean survival duration in responders was 15.2 months and in nonresponders was 8.9 months ($P \leq 0.01$). Hematologic and subjective toxic effects are discussed. [Cancer Treat Rep 68:647–650, 1984]

The search for effective therapy for advanced colorectal carcinoma has been ongoing since the development and clinical application of 5-FU > 20 years ago. No single agent has been shown to be consistently more effective than 5-FU, which has provided response rates of 15%–20% when administered by the iv route (1–3). Studies of drug combinations including 5-FU have yielded varying results (4,5). While one colorectal study of a combination of 5-FU, mitomycin (MMC), and methotrexate (MTX) had only a 5% (one response among 20 patients) partial response rate (6), other drug combinations have shown response rates greater than that of 5-FU alone (7–10).

This study was undertaken to investigate the value of a combination of 5-FU, MMC, MTX, and vincristine (VCR). Both 5-FU and MMC have definite activity against metastatic colorectal carcinoma. Scattered reports have suggested a role for the use of MTX and VCR in this disease (4,6).

Our initial experience with this combination was favorable and led to further evaluation (11).

PATIENTS AND METHODS

Fifty-three patients with advanced metastatic colorectal carcinoma were selected for treatment with 5-FU, MMC, VCR, and MTX. Eligibility requirements included: (*a*) expected survival of at least 1 month without treatment; (*b*) no radiation therapy, surgery, or cytotoxic chemotherapy for at least 2 weeks prior to treatment; (*c*) a known area of malignant disease including carcinoembryonic antigen (CEA) that could be measured to serve as an objective indicator of response; (*d*) normal renal function as reflected by a normal BUN and creatinine (BUN ≤ 25 mg/100 ml; creatinine ≤ 1.5 mg/100 ml); and (*e*) adequate or mildly compromised bone marrow reserve (wbc count ≥ 3000/mm³ and platelet count ≥ 100,000/mm³).

Twenty-one patients were men and 32 were women. Their ages ranged from 24 to 83 years (median, 65.0; mean, 63.4). Thirty-five patients were diagnosed as having recurrent disease, with disease-free time since surgery ranging from 2 to 72 months (mean, 22.5). Eighteen patients had metastatic disease at initial diagnosis. The sites of metastases at the start of treatment were: liver only (20 patients); lung only (five); soft tissue only, not liver or lung (eight); liver and lung (12); lung and soft tissue (two); liver and soft tissue (four); and liver, lung, and soft tissue (two). Two patients developed bone metastases as their disease progressed. All metastatic sites were confirmed by biopsy, x-ray, or scan.

Performance status was measured in each patient prior

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to start of chemotherapy. Eighteen patients had grade 0 (not impaired), 21 had grade 1, and 14 had grades > 1.[5]

Twelve patients had received prior therapy. Eight patients had had prior radiation therapy only. One patient had had prior chemotherapy with 5-FU only, and one had had prior chemotherapy with 5-FU and MTX. Two patients had received prior radiation therapy and chemotherapy with 5-FU.

All patients were treated as ambulatory outpatients.

Prior to treatment, BUN, creatinine, SGOT, alkaline phosphatase, bilirubin, LDH, cbc, platelet count, and CEA were obtained for all patients. During treatment, cbc and platelet count were obtained every 2 weeks, and a CEA and chemistry screen were obtained prior to each course.

The initial course of chemotherapy consisted of 10 mg/m^2 of MMC on Day 1 only; 300 mg/m^2 iv of 5-FU on Days 1-5; 0.75 mg/m^2 of VCR on Days 1 and 5; and 8.0 mg/m^2 iv of MTX on Days 1 and 5. Subsequent courses were repeated every 4-5 weeks, depending on patient tolerance and hematologic recovery. MMC was repeated only every other course. All drugs were given by iv push through a flowing iv. No particular sequence of drug administration was observed. Dose levels were raised or lowered as observed bone marrow toxicity permitted; no more than 1.0 mg/m^2 of VCR was administered per dose. MMC was withheld for 4 weeks if the platelet count was < 100,000/mm^3 and was reduced by 20% if the platelet count was < 150,000/mm^3 when the next course was due. MTX and MMC were withheld if creatinine was > 2.0 mg/100 ml or BUN was > 30 mg/100 ml. VCR was withheld if significant neuropathy developed.

A complete response (CR) was defined as the disappearance of all measurable tumor. A partial response (PR) was defined as a reduction in the sum of perpendicular diameters of the most clearly measurable area of known cancer by at least 30% for lesions ≥ 5.0 cm in diameter and at least 50% for lesions < 5.0 cm. Six of the ten responders who had hepatic involvement only had lesions > 4.0 cm in diameter on radionuclide scans. The remaining four patients with liver involvement had malignant hepatomegaly, with the liver extending > 5.0 cm below the right costal margin. In order for the latter group to be included as responders, it was necessary that the liver return to normal size on physical examination and that improvement in liver function tests be observed as well. A 50% decrease in CEA, when no other measurable disease was present, could only be considered a PR (two patients). There could be no increase of any known lesion and no new lesions. Responses less than PR as defined above were all considered as no response. When multiple lesions were present (ie, liver and lung), both measurable lesions had to decrease as previously indicated to be considered a response.

[5]Southwest Oncology Group criteria for evaluation of performance status.

RESULTS

Three patients (5.7%) achieved CR and 20 patients (37.7%) achieved PR. Thirty patients had no response. The CR median duration was 6.0 months (mean, 13.3; range, 3.5-31.0). Overall PR median duration was 7.0 months (mean, 7.8; range, 2.0-36.0). The difference in observed mean survival duration between responders and nonresponders was highly significant ($P \leq 0.01$). Mean survival duration for responders was 15.2 months (median, 14.5) and ranged from 3.5 to 28.0. Mean survival duration for nonresponders was 8.9 months (median, 6.0) and ranged from 2.0 to 21.0. The 1-year actuarial survival rate for responders (60.9%) was significantly higher ($P \leq 0.05$) than the 1-year rate for nonresponders (33.3%). Response rates in each site of metastasis were: 50% (liver only, ten of 20 patients); 0% (lung only, none of five); 62.5% (soft tissue only, five of eight); 50% (liver and lung, six of 12); 50% (lung and soft tissue, one of two); 25% (liver and soft tissue, one of four); and 0% (liver, lung, and soft tissue, none of two).

All metastatic sites exhibited some degree of response to chemotherapy except lung only (five patients) and lung, liver, and soft tissue (two). A larger sampling of patients with lung only and liver, lung, and soft tissue metastases would be needed to determine the effective response rate of these metastatic sites to chemotherapy.

Four of 12 patients (33.3%) with prior treatment responded to chemotherapy: one had had prior radiation therapy, one had received prior 5-FU, one had had prior 5-FU and MTX, and one had had prior radiation therapy and 5-FU. Patients from each prior treatment category responded. This would suggest that prior therapy does not preclude response to this treatment combination.

A decrease in CEA of > 50% was used in two patients as the sole indication of response. Both patients had extensive intra-abdominal disease which was documented at surgery and not measurable by x-ray, scan, or direct observation. Both patients had CEA ≥ 10 ng/ml prior to initiation of therapy and both experienced weight gain, increased appetite, and activity in association with a decrease in CEA. Twelve other responders had CEA measured prior to and during treatment. Of these patients, ten experienced a ≥ 50% decrease in CEA in conjunction with a decrease in the size of the measurable lesion(s).

Responders had a significantly better performance status than nonresponders ($P \leq 0.025$) at the time treatment was initiated. Twelve of 21 men (57.1%) and 11 of 32 women (34.4%) responded, but this difference was not significant. Responders with recurrent disease had a mean time to recurrence of 28.8 months, while nonresponders had a mean time to recurrence of 18.7 months. However, the difference was not significant.

Response did not appear to be age-related. Responders were not significantly younger than nonresponders. The

TABLE 1.—Hematologic toxicity of 5-FU, MMC, VCR, and MTX

Toxic effect, grade	No. (%) of patients with toxic effect	
	Responders	Nonresponders
Leukopenia (cells/mm^3)		
0 (≥ 4000)	5 (21.7)	8 (26.7)
1 (3000–3999)	2 (8.7)	8 (26.7)
2 (2000–2999)	10 (43.5)	9 (30.0)
3 (1000–1999)	5 (21.7)	4 (13.3)
4 (< 1000)	1 (4.3)	1 (3.3)
Thrombocytopenia (cells/mm^3)		
0 (≥ 100,000)	11 (47.8)	17 (56.7)
1 (75,000–99,999)	2 (8.7)	4 (13.3)
2 (50,000–74,999)	2 (8.7)	4 (13.3)
3 (25,000–49,999)	4 (17.4)	2 (6.7)
4 (< 25,000)	4 (17.4)	3 (10.0)

median age of responders was 61.0 years (mean, 61.0), and the median age of nonresponders was 65.0 years (mean, 65.2).

Hematologic toxicity was graded by severity. Table 1 shows the leukopenia and thrombocytopenia experienced by patients.

Leukopenia of grade 1 or greater was experienced by 78.3% of responders, and thrombocytopenia of grade 1 or greater was experienced by 52.2%. Leukopenia of grade 1 or greater was experienced by 73.3% of nonresponders, and thrombocytopenia of grade 1 or greater was experienced by 43.3%. There was no significant difference in hematologic toxicity between responders and nonresponders.

Overall, 56.6% of patients experiencd nausea; 30.2%, anorexia; 18.9%, diarrhea; 17.0%, vomiting; 17.0%, lethargy; 9.4%, abdominal cramping; 1.9%, leg cramping; 9.4%, mucositis; 9.4%, edema; 5.7%, alopecia; and 3.8%, epistaxis. Forty-one patients (77.4%) experienced two or more of these side effects. Responders experienced 39.8% of side effects and nonresponders experienced 60.2%.

There were three toxicity-related deaths: one from septicemia secondary to leukopenia, one due to local infection secondary to extravasation of MMC, and one due to hemorrhage secondary to thrombocytopenia.

DISCUSSION

The treatment of metastatic colorectal carcinoma remains unsatisfactory. This particular drug combination was used initially by us in 1977. The individual drugs were selected because recent reports had suggested that VCR was effective (1,4); both MMC and 5-FU have well-documented but limited effectiveness. MTX alone had previously been used by one of the authors with limited success. The possible synergy of MTX and 5-FU combinations had not been reported at that time (12,13). For these reasons, no particular sequence of drug administration was used in this group of patients. However, the recent reports indicating possible synergy between MTX and 5-FU have used higher doses of MTX, and the administration of 5-FU was delayed by several hours. In all of our patients the drugs were administered in rapid sequence without any planned delay. This would seem to indicate that the recently reported synergy between MTX and 5-FU observed with sequential administration was not a factor in this group of patients.

Given the relatively low toxicity of this drug combination and the observed effectiveness, we plan further studies with these drugs. Our experience would suggest that the MMC could be administered with every course of therapy instead of every other course, without an appreciable increase in toxicity.

REFERENCES

1. MOERTEL C. Clinical management of advanced gastrointestinal cancer. Cancer 36:675, 1975.
2. LIVINGSTON RB, and CARTER SK. Single Agents in Cancer Chemotherapy. IFI/Plenum, 1970.
3. CARTER SK, BAKOUSKI MT, HELLMAN K, eds. Chemotherapy of Cancer. John Wiley and Sons, 1981.
4. FALKSON G, VAN EDEN EB, ET AL. Fluorouracil, imidazole carboxamide dimethyl triazeno, vincristine and bis chloroethyl nitrosourea in colon cancer. Cancer 33:1207–1209, 1974.
5. LOKICH JJ, and SKARIN AT. Combination therapy with 5-fluorouracil (5-FU; NSC-19893) and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) for disseminated gastrointestinal carcinoma. Cancer Chemother Rep 56:653–657, 1972.
6. BIRAM H, and SULKES A. FMM: 5-fluorouracil (f), methotrexate (MTX), and mitomycin C (MMC). A phase II study in advanced colorectal cancer. Proc Am Assoc Cancer Res and ASCO 21:291, 1980.
7. MAKEL JA. Combination chemotherapy and scheduling methotrexate and mitomycin C against murine tumors. Proc Am Assoc Cancer Res and ASCO 21:291, 1980.
8. SOLAN A, ET AL. 5-Fluorouracil (FU) and methotrexate (MTX) in combination for colorectal cancers: unacceptable toxicity when intermediate dose MTX precedes FU by 4 hours. Proc Am Assoc Cancer Res and ASCO 21:335, 1980.
9. DRAPKIN R, ET AL. Sequential methotrexate (MTX) and 5-fluorouracil (5-FU) in adenocarcinoma of the colon and rectum. Proc Am Assoc Cancer Res and ASCO 22:435, 1981.
10. HERRMAN R, ET AL. Sequential methotrexate (MTX) and 5-fluorouracil (FU) in colorectal adenocarcinoma. Results of a pilot study.

Proc Am Assoc Cancer Res and ASCO 22:457, 1981.
11. BECK TM, HART NE, SMITH CE, ET AL. Treatment of colorectal carcinoma with combination chemotherapy. Proc Am Assoc Cancer Res and ASCO 21:324, 1980.
12. TISMAN G, and WU SJG. Effectiveness of intermediate-dose methotrexate and high-dose 5-fluorouracil as sequential combination chemotherapy in refractory breast cancer and as primary therapy in metastatic adenocarcinoma of the colon. Cancer Treat Rep 64:829–835, 1980.
13. WEINERMEN B, SCHACTER B, SCHIPPER H, ET AL. Sequential methotrexate and 5-FU in the treatment of colorectal cancer. Cancer Treat Rep 66:1553–1555, 1982.

Effects of Amphotericin B With Combination Chemotherapy on Response Rates and on Survival in Non-Small Cell Carcinoma of the Lung[1,2]

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Thirty-seven patients with non-small cell carcinoma of the lung were randomized to receive doxorubicin, lomustine, hexamethylmelamine, and methotrexate (ACHM) or ACHM plus amphotericin B (AMB). The complete plus partial response rate was 39% for ACHM plus AMB, compared to 23% for ACHM alone. However, the median duration of complete or partial response was only 3.0 months with ACHM plus AMB, compared to 7.0 months with ACHM. Most importantly, median survival was only 4.0 months with ACHM plus AMB, compared to 8.0 months with ACHM ($P = 0.08$; two-tail test). Myelosuppression was enhanced by the addition of AMB. Although AMB has biological and antitumor activity in certain clinical circumstances, it does not appear to have a meaningful role in palliative therapy of patients with non-small cell bronchogenic carcinoma. [Cancer Treat Rep 68:651–654, 1984]

Amphotericin B (AMB) plus carmustine is synergistic in murine AKR leukemia (1-3) because of immunostimulation by AMB (4-8). In addition, AMB increases the permeability of eukaryotic cells to drugs and macromolecules (9-11) and increases the uptake of antitumor agents in tumor cells (12,13). Our prior clinical studies of AMB plus carmustine in lung cancer suggested an increased response rate (14,15). Therefore, we conducted a randomized prospective study of combination chemotherapy utilizing four of the most active chemotherapeutic agents in patients with metastatic non-small cell bronchogenic carcinoma (NSBC).

METHODS

Patients with nonresectable NSBC with a Karnofsky performance status ≥ 30% were eligible for the trial. No patient had received prior chemotherapy.

All patients received chemotherapy with doxorubicin, lomustine, hexamethylmelamine, and methotrexate (ACHM) every 8 weeks: doxorubicin (40 mg/m^2 iv) on Days 1 and 29; carmustine (70 mg/m^2 orally) on Day 2; hexamethylmelamine (160 mg/m^2 orally in four divided doses) on Days 2-15 and 30-43; and methotrexate (40 mg/m^2 in four divided doses orally) on Days 2, 9, 30, and 37. The doses were reduced in a standard fashion according to myelosuppression or gastrointestinal or neurological toxic effects observed. Patients were randomized to receive the following doses of either ACHM alone or ACHM with concurrent AMB: 7.5 mg/m^2 on Day 0 (the day prior to doxorubicin); 30 mg/m^2 on Day 1; and 30 mg/m^2 on Day 29 of each 8-week cycle of therapy. AMB was given in 250 ml of 5% dextrose in water iv over 6 hours, with diphenhydramine, acetaminophen, and hydrocortisone when necessary to control chills or fever. When severe chills were observed, meperidine (25-50 mg) was given iv.

A complete response was defined as the complete disappearance of all objective evidence of disease and the attainment of normal performance status. A partial response was defined as a > 50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions. Stable disease was defined as less than a partial response, with no enlargement of measured tumors by > 25% in the sum of the products of the diameters and no new lesions appearing during therapy. All other patients were defined as having tumor progression. To have any response, maximal response must have been observed on at least two successive occasions at least 4 weeks apart (a partial response of brief duration of ≤ 1 month was classified as stable or progressive disease).

Tabular data were compared using the Fisher exact test or the binomial distribution. Disease-free and total sur-

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vival curves were constructed using the Kaplan-Meier technique (17), and curves were compared by the modified Wilcoxon test (18) or Mantel-Cox test (16).

RESULTS

Of the 37 patients admitted to this trial, six were in-

TABLE 1.—Patient characteristics*

	ACHM + AMB	ACHM	Total
Patients	21	16	37
Cell type			
Adenocarcinoma	12	8	20
Squamous cell carcinoma	5	5	10
Large cell carcinoma	4	3	7
Sex			
Male	17	9	26
Female	4	7	11
Prior radiation therapy			
Radiation therapy	12	9	21
No radiation therapy	9	7	16
Extent of disease at entry			
Limited	7	5	12
Extensive	14	9	23
Staging incomplete	0	2	2
Performance status at entry			
> 90	6	2	8
70-89	5	6	11
50-69	8	3	11
< 50	1	4	5
Unknown	1	1	2
Evaluable for response			
Evaluable	18	13	31
Not evaluable	3	3	6
No. of courses given			
1	7	4	11
2	6	3	9
3	2	4	6
> 3	3	2	5
Response			
Complete	0 } 39%	1 } 23%	
Partial	7 }	2 }	
Stable disease	6	7	
Disease progression	5	3	
Duration of response (mos)			
Complete or partial	3.0	7.0	
Stable	2.5	5.0	
Survival (mos)	4.0	8.0†	
Toxicity			
Granulocyte count nadir (cells/mm^3)‡			
< 500	9 (56%)	3 (23%)	
500-1500	5 (31%)	9 (69%)	
> 1500	2 (13%)	1 (8%)	
Platelet count nadir (cells/mm^3)§			
< 25,000	8 (47%)	1 (8%)	
25,000-90,000	7 (41%)	6 (46%)	
> 90,000	2 (12%)	6 (46%)	

*Unless otherwise specified, values = No. of patients.
†$P = 0.08$, 2-tail test.
‡Chi-square test, ACHM + AMB vs ACHM, $P = 0.12$ (not significant).
§Chi-square test, ACHM + AMB vs ACHM, $P = 0.03$.

evaluable due to insufficient data, patient noncompliance, or inadequate follow-up. Patient characteristics were similar (table 1).

The addition of AMB produced a response rate of 39%, compared to a 23% response rate with ACHM alone (P = 0.30, Fisher exact test). (With the small size of this study, the power to detect the observed difference is only 25%. However, to establish such a difference with a power of 90% and significant level of 5%, a total of 280 patients would have been required.) Despite the trend towards a higher response rate with the AMB plus ACHM arm, the median duration of response with the ACHM plus AMB arm was only 3 months (1, 2, 3, 5+, 9, and 13), while with the ACHM arm it was 7 months (4, 7, and 18+). Similarly, the median duration of stable disease was 2.5 months with ACHM plus AMB (2, 2, 2, 3+, 4, and 6 months), while it was 5 months with ACHM (3, 3+, 4, 5, 5, 6, and 6 months). Survival favored the ACHM-alone arm (fig 1). While median survival was only 4.0 months with ACHM plus AMB, it was 8.0 months with ACHM (significant at P = 0.079 by two-tail generalized Wilcoxon test of Gehan, and P = 0.086 for two-tail test by the generalized Savage test of of Mantel-Cox).

Toxicity was more severe with ACHM plus AMB (table 1). The dose-limiting toxic effect was myelosuppression. Fifty-six percent of the patients receiving AMB had a granulocyte count nadir < 500/mm^3, and 47% had a platelet count nadir < 25,000/mm^3. By comparison, only 23% of patients treated with ACHM had a granulocyte count nadir of that severity (P = 0.12), and only 8% treated with ACHM had a platelet count nadir of that severity (P = 0.03). No treatment-related sepsis or drug-related death occurred in either arm. In addition, chills and fever were observed only with the AMB treatment program. Nausea and vomiting, stomatitis, and diarrhea were equal with both arms. ECG changes were reported in three patients on the ACHM plus AMB arm of the study.

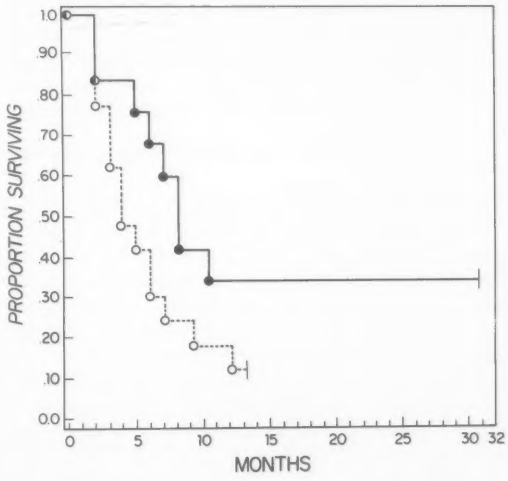

FIGURE 1.—Survival of patients according to treatment regimen. Solid circles and lines represent ACHM. Open circles and interrupted lines represent ACHM + AMB. Vertical bars represent patients still alive.

DISCUSSION

This randomized prospective study has indicated that although AMB tends to have a favorable effect on response rates to ACHM in patients with metastatic NSBC, an unfavorable effect is produced on survival and myelosuppression is increased (as well as stomatitis and diarrhea). Since our results in the chemotherapy arm were similar to those of comparable prior combination chemotherapy studies in NSBC (19-24), our conclusions are likely to be valid. The reasons for the shorter survival with AMB are probably the shorter durations of response and times to progression in patients with stable disease. This suggests that AMB may actually hasten the emergence of resistant clones within the tumor. Therefore, we do not recommend the addition of AMB, used as initial palliative therapy in this disease, to any antitumor chemotherapeutic regimen. However, since further experimental studies in leukemic mice have shown that prolonged AMB administration leads to more marked chemosensitization (F. Valeriote, personal communication), further exploratory trials of 7-14-day AMB prior to chemotherapy may be warranted. Although AMB has a clear-cut biological effect in experimental animals, and although the addition of AMB to chemotherapy can produce antitumor responses in patients previously resistant to the same chemotherapy used alone (25,26), there appears to be no conclusive role at present for the use of AMB in NSBC.

REFERENCES

1. MEDOFF G, SCHLESSINGER D, and KOBAYASHI GS. Polyene potentiation of antitumor agents. JNCI 50:1047, 1973.
2. MEDOFF G, VALERIOTE F, LYNCH RG, ET AL. Synergistic effect of amphotericin B and 1,3-bis (2-chloroethyl)-1-nitrosourea against a transplantable AKR leukemia. Cancer Res 34:974, 1974.
3. MEDOFF G, VALERIOTE F, RYAN J, ET AL. Response of transplanted AKR leukemia to combination therapy with amphotericin B and 1, 3-bis (2-chloroethyl)-1-nitrosourea: dose and schedule dependency. JNCI 58:949, 1977.
4. BLANKE TJ, LITTLE JR, SHIRLEY SF, ET AL. Augmentation of murine immune responses by amphotericin B. Cell Immunol 38:180-190, 1977.
5. BLANKE TJ, LYNCH R, and LITTLE JR. Mechanisms of antitumor effects of amphotericin B. Surg Forum 26:126, 1975.
6. LITTLE JR, BLANKE TJ, VALERIOTE F, ET AL. Immunoadjuvant and antitumor properties of amphotericin B. Prog Cancer Res Ther 7:381-387, 1978.
7. SHIRLEY SF, and LITTLE JR. Immunopotentiating effects of amphotericin B: enhanced contact sensitivity in mice. J Immunol 123:2878-2882, 1979.

8. SHIRLEY SF, and LITTLE JR. Immunopotentiating effects of amphotericin B: enhanced in vitro proliferative responses of murine lymphocytes. J Immunol 123:2882-2889, 1979.
9. KUMAR BV, and MEDOFF G. Uptake of *Escherichia coli* DNA into HeLa cells enhanced by amphotericin B. Nature 250:323-325, 1974.
10. KWAN CN, MEDOFF G, KOBAYASHI GS, ET AL. Potentiation of the antifungal effects of antibiotics by amphotericin B. Antimicrob Agents Chemother 2:61-65, 1972.
11. MEDOFF G, COMFORT M, and KOBAYASHI GS. Synergistic action of amphotericin B and 5-fluorocytosine against yeast-like organisms. Proc Soc Exp Biol Med 138:571-574, 1971.
12. NAKAZAWA I, OUCHI E, ISHIDA N, ET AL. Effects of amphotericin B on the incorporation of neocarzinostatin into human gastric cancerous tissues. Tokoho J Exp Med 124:97, 1978.
13. PRESANT CA, and CARR D. Amphotericin B (Fungizone^{R}) enhancement of nitrogen mustard uptake by human tumor cells. Biochem Biophys Res Commun 93:1067-1073, 1980.
14. PRESANT CA, HILLINGER S, and KLAHR C. Phase II study of BCNU with amphotericin B in bronchogenic carcinoma. Cancer 45:6-10, 1980.
15. PRESANT CA, KLAHR C, OLANDER J, ET AL. Amphotericin B plus 1,3-bis (2-chloroethyl)-1-nitrosourea in advanced cancer: phase I and preliminary phase II results. Cancer 38:1917-1921, 1976.
16. MANTEL N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163-170, 1966.
17. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.
18. GEHAN EA. A generalized Wilcoxon test for comparing arbitrarily singly censored samples. Biometrika 52:203-233, 1965.
19. MILSTEIN D, and ROBINSON E. Four-drug combination chemotherapy in advanced lung cancer: methotrexate, doxorubicin, cyclophosphamide and CCNU. Cancer 48:2358-2363, 1981.
20. SELAWRY O. The role of chemotherapy in the treatment of lung cancer. Semin Oncol 1:259-272, 1974.
21. LIVINGSTON RB. Combination chemotherapy of bronchogenic carcinoma. I. Non-oat cell. Cancer Treat Rev 4:153-165, 1977.
22. SELAWRY OS, and HANSEN HH. Lung cancer. *In* Cancer Medicine (Holland JF, and Frei E, eds). Philadelphia, Lea and Febiger, 1982, p 1709.
23. CHAHINIAN AP, MANDEL EM, HOLLAND JF, ET AL. MACC (methotrexate, adriamycin, cyclophosphamide and CCNU) in advanced lung cancer. Cancer 43:1590-1597, 1979.
24. VOGL SE, MEHTA CR, and COHEN MH. MACC chemotherapy for adenocarcinoma and epidermoid carcinoma of the lung. Low response rate in a cooperative group study. Cancer 44:864-868, 1979.
25. PRESANT CA, KLAHR C, and SANTALA R. Amphotericin B induction of sensitivity to adriamycin, 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU, NSC # 409962) plus cyclophosphamide in human neoplasia. Ann Intern Med 86:47-51, 1977.
26. PRESANT CA, and GARRETT S. Drug resistance: reversal by amphotericin B. Proc Am Assoc Cancer Res and ASCO 21:138, 1980.

Long-Term Effects of Dichloromethylene Diphosphonate in Patients With Osteolytic Bone Metastases and Coincident Primary Hyperparathyroidism[1,2]

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Dichloromethylene diphosphonate (Cl_2MDP), an inhibitor of osteoclast-mediated bone resorption, lowers serum calcium in hypercalcemia associated with malignancies and with primary hyperparathyroidism. We have evaluated the effectiveness of Cl_2MDP in three patients who had multiple osteolytic bone metastases due to breast cancer and coincident primary hyperparathyroidism and who refused neck exploration. Cl_2MDP was added to the tamoxifen treatment and was given orally at a dose of 1600 mg/day for 12 months. All patients had a reduction in serum calcium level which was accompanied by a decline in the fasting urinary calcium and hydroxyproline excretion. Administration of Cl_2MDP was not associated with any changes in parathyroid hormone levels. New bone metastases were observed neither during the treatment nor in the follow-up period. No side effects were observed. [Cancer Treat Rep 68:655–657]

Diphosphonates inhibit the action of the osteoclast, the cell responsible for bone resorption (1). Dichloromethylene diphosphonate (Cl_2MDP) has reduced the biochemical indexes of bone resorption in malignancies and primary hyperparathyroidism (PHPT) (2–5). The purpose of the present study was to investigate whether Cl_2MDP, used as supportive long-term treatment, is effective in patients who have both osteolytic bone metastases and coincident PHPT. We were interested in knowing whether the drug reduces osteoclast function in cases in which the cell mediates increased bone resorption and simultaneously is a target cell for enhanced action of parathyroid hormone (PTH).

PATIENTS AND METHODS

Three women with stage II breast cancer were studied. Patient 1 was 64 years of age, Patient 2 was 71, and Patient 3 was 67. Two to 3 years after primary therapy, multiple bone metastases (cranial, costal, vertebral, and pelvic) occurred, which were verified by x-ray, isotope scanning, and biopsy. Because the metastases were osteolytic, the biochemical indexes of bone resorption were determined. All three patients had simultaneous occurrence of hypercalcemia, hypophosphatemia, hyperchloremia, and a high level of serum PTH which suggested a coincident PHPT. Each patient had normal creatinine clearance. PHPT was supported by additional factors. Patient 2 had received x-ray therapy because of tuberculous lymphadenitis of the neck 50 years previously. Patient 3 had passed kidney stones. Subcortical bone resorption in the phalanges and distal clavicles had been observed roentgenographically in Patient 1. In two patients, ultrasonography revealed adenoma (9 × 10 and 13 × 14 mm) in the right inferior parathyroid gland. Patient 2 had enlargement of the upper two parathyroid glands, which were considered to be hyperplastic. In addition, three thyroid nodules were found. All three patients refused neck exploration.

Metastatic receptor-positive breast cancer of each patient was treated with tamoxifen. The level of serum calcium showed no changes during this treatment. Since no calcification of the bone metastases was seen after 6 months of tamoxifen therapy and since the bone lesions continued to be painful, Cl_2MDP therapy was added to the tamoxifen treatment. All patients continued to receive tamoxifen at a dose of 20 mg twice daily. Cl_2MDP (donated by Star Pharmaceutical Co, Tampere, Finland) was given orally at a dose of 1600 mg/day for 12 months. None of the patients had any soft tissue metastases. The assessment of the intensity of bone pain was based on the daily consumption of analgesic drugs. Serum calcium, albumin, creatinine, phosphate, alkaline phosphatase, and PTH were measured every month. Fasting urine was col-

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lected over 2 hours after a 12-hour fast and was measured for calcium, creatinine, and hydroxyproline. Bone scans (^{99m}Tc-MDP) were done before therapy and repeated every 3 months during 2 years. The bone lesions were also registered and followed radiographically. Laboratory methods are described elsewhere (3).

RESULTS

None of the patients reported any side effects, and there was no evidence of hematological toxicity.

During the Cl_2MDP treatment the patients had no pain, and the daily consumption of analgesic drugs stopped. The serum calcium decreased but remained mildly elevated (fig 1). The high fasting urinary hydroxyproline/creatinine (OHP/Cr) and calcium/creatinine (Ca/Cr) ratios also declined to the normal or low ranges in all patients except Patient 2, whose urinary Ca/Cr ratio decreased but remained elevated (fig 1). The pretreatment levels of serum phosphate and alkaline phosphatase persisted at the lower and upper limits of the normal range, respectively (fig 1). Serum PTH level was as high as before the Cl_2MDP treatment. No reduction of previously existing osteolytic lesions was observed, but a calcification of some lesions was seen after 12 months' therapy. New bone metastases were observed neither in the bone scans during the Cl_2MDP treatment period nor in the follow-up period of 1 year. The formation of kidney stones stopped in Patient 3.

DISCUSSION

It is likely that the hypercalcemia in these patients was due to PHPT rather than to osteolytic bone metastases, since the levels of serum PTH were high (6). It is unlikely that the cancer was producing an ectopic PTH-like substance (7). Further, by definition, there are no bone metastases in ectopic hyperparathyroidism (8). Tamoxifen therapy may increase serum calcium levels; however, the patients had hypercalcemia before tamoxifen therapy (9). In addition, it is well-known that PHPT may develop after radiation therapy of the neck; kidney stones are rare in malignant hypercalcemia, and characteristic bone changes of PHPT are absent in ectopic hyperparathyroidism (8,10). Thus, each of the patients most likely had coexistent PHPT, in which diagnosis was supported by the ultrasonography findings.

A fall of serum calcium levels is likely to be mediated

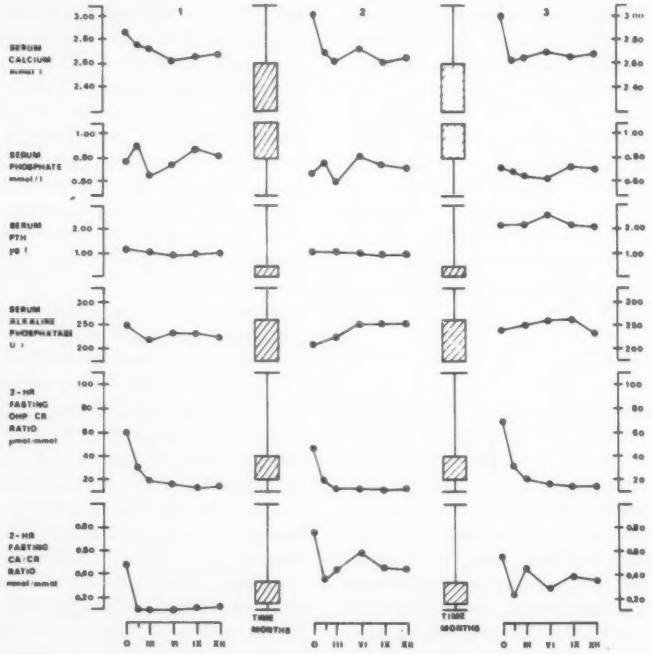

FIGURE 1.—Changes in serum calcium, phosphate, PTH, and alkaline phosphatase levels and in the fasting urinary OHP/Cr and Ca/Cr ratios during 12 mos of Cl_2MDP treatment in 3 women with osteolytic bone metastases due to breast cancer and coincident PHPT. Shaded vertical bars represent the normal ranges.

by inhibitory action of the Cl_2MDP on the osteoclast, thus impairing the increased rate of skeletal mobilization of calcium. Similarly, a decrease in the fasting urinary OHP/Cr and Ca/Cr ratios supports this mechanism of action for Cl_2MDP. The reduced filtered load of calcium during the treatment period is likely to contribute to the lowered urinary calcium excretion which has been observed both in PHPT and in metastatic bone loss (2–5).

The decline of serum calcium was not accompanied by a decrease in the PTH level. These results are consistent with recent observations made by Douglas et al (4) and by Shane et al (5), which showed no significant correlation between changes in serum PTH and serum calcium levels in patients with PHPT treated with Cl_2MDP. The observations support the concepts that the abnormal tissue responsible for PHPT is not as finely regulated by calcium as normal parathyroid tissue (5).

The benefit of Cl_2MDP treatment included reduction of bone pain, delay of bone lesion extension, and prevention of new bone destruction. These effects of decreased bone resorption persisted beyond the period of drug administration. Cl_2MDP may alter the integrity of the bone remodeling unit, possibly by binding to hydroxyapatite and by impairing the function of the osteoclast, thus causing prolonged effects (1).

From these results we conclude that Cl_2MDP, given as supportive therapy, is effective in reducing hypercalcemia and osteolytic bone destruction in patients with breast cancer and coincident PHPT. This may be important for patients who have refused neck exploration and for whom surgery is contraindicated or unsuccessful.

REFERENCES

1. FLEISCH H, and FELIX R. Diphosphonates. Calcif Tissue Int 27:91–94, 1979.
2. CHAPUY MC, MEUNIER PJ, ALEXANDRE CM, ET AL. Effects of disodium dichloromethylene diphosphonate on hypercalcemia produced by bone metastases. J Clin Invest 65:1243–1247, 1980.
3. ELOMAA I, BLOMQVIST C, GRÖHN P, ET AL. Long-term controlled trial with diphosphonate in patients with osteolytic bone metastases. Lancet 1:146–149, 1983.
4. DOUGLAS DL, DUCKWORTH T, RUSSELL RGG, ET AL. Effect of dichloromethylene diphosphonate in Paget's disease of bone and in hypercalcemia due to primary hyperparathyroidism or malignant disease. Lancet 1:1043–1047, 1980.
5. SHANE E, BAQUIRAN DC, and BILEZIKIAN JP. Effects of dichloromethylene diphosphonate on serum and urinary calcium in primary hyperparathyroidism. Ann Intern Med 95:23–27, 1981.
6. APICHATI U, AVRAAM A, GARDNER B, ET AL. Primary hyperparathyroidism and breast cancer. Am J Med 61:136–139, 1976.
7. HIRSHORN JE, VRHOVSEK F, and POSEN S. Carcinoma of the breast associated with hypercalcemia and the presence of parathyroid hormone like substances in the tumor. J Clin Endocrinol Metab 48:217–221, 1979.
8. BLACKMAN MR, ROSEN SW, and WEINTRAUB BD. Ectopic hormones. Adv Intern Med 23:85–113, 1978.
9. LEGHA SS, POWELL K, BUZDAR AU, ET AL. Tamoxifen-induced hypercalcemia in breast cancer. Cancer 47:2803–2806, 1981.
10. CHRISTENSSON T. Hyperparathyroidism and radiation therapy. Ann Intern Med 89:216–217, 1978.

Cisplatin-Induced Autonomic Neuropathy[1]

Calvin S. Rosenfeld* and Lawrence E. Broder[2]

A 57-year-old man with bronchogenic squamous cell carcinoma developed an autonomic neuropathy following treatment with a chemotherapy combination which included cisplatin. The autonomic neuropathy resolved following discontinuation of the cisplatin and treatment with dextroamphetamine. The following case represents a patient with bronchogenic carcinoma treated with cyclophosphamide, doxorubicin, and cisplatin, who developed a severe autonomic neuropathy following chemotherapy administration. This is the first report of cisplatin-associated autonomic neuropathy to appear in the literature. [Cancer Treat Rep 68:659–660, 1984]

CASE REPORT

A 57-year-old white male developed a cough and chest pain in August 1981. A chest x-ray revealed a mass in the left upper lobe. The past medical history was unremarkable and he was on no medication. He was a smoker for > 60 pack years but denied alcohol abuse. There was no carcinogenic or neuropathic exposure otherwise. Physical examination revealed normal vital signs, with a blood pressure of 130/90 mm Hg and a pulse rate of 80/minute. The abnormal findings were limited to the chest, which revealed asymmetrical movement with reduced excursion on the left, tubular breath sounds over the left lung field, and diffuse expiratory wheezing bilaterally. The neurological assessment of cranial nerves, motor/sensory systems, and reflexes was normal, as was the remainder of the physical examination. A bronchoscopy revealed partial obstruction of the left upper lobe bronchus. A mediastinoscopy was positive for moderately differentiated squamous cell carcinoma (stage III). A cbc, blood chemistries (SMA-18), and bone and liver scans were within normal limits. The patient developed postobstructive pneumonitis, and radiation therapy to the primary and mediastinum was begun. Following completion of therapy, a partial response was obtained as determined by tumor measurements, reaeration of the left lung, and resolution of the postobstructive pneumonitis.

The patient did well until March 1982, when he developed recurrent tumor in the left upper lobe. Cytopathology obtained at bronchoscopy confirmed the diagnosis and chemotherapy was initiated with cyclophosphamide (500 mg/m^2), doxorubicin (50 mg/m^2), and cisplatin (60 mg/m^2) (CAP), all given iv every 3 weeks. The patient tolerated the chemotherapy initially, but 48 hours later complained of severe nausea, vomiting, and dizziness. The nausea resolved but the dizziness progressed and was posturally related, preventing the patient from walking or standing erect. Physical examination revealed a supine blood pressure of 100/60 mm Hg and a pulse of 90/minute; while the patient was erect, the readings were 80/50 mm Hg and 100/minute, respectively. A cbc at this time was within normal limits, as were the serum electrolytes. However, the BUN was 37 mg/dl and hypovolemia was considered the etiology of his dizziness. The patient was hydrated with iv fluids and discharged, to be followed on an outpatient basis. However, his complaint of postural dizziness persisted. A second cycle of CAP was administered on schedule and similar complaints were noted. In addition to his dizziness, the patient reported a dry mouth, frequent loose stools, and an ice-cold sensation in his feet. One week after Cycle II therapy, the clinical picture now included ataxia, paresthesias, and poikilothermia. He was admitted to the hospital for neurological evaluation.

The patient's blood pressure continued to demonstrate postural hypotension (110/70 supine and 80/50–60 palpable when erect) associated with pulse rates recorded at 72/minute (supine) and 80/minute (erect). The neurological evaluation revealed diminished reflexes (1+ in the upper extremities, trace at the knees, and absent at the ankles) and absent plantar responses. The sensory examination was also abnormal, with decreased light touch and

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pin prick in a stocking distribution to mid-calf. The vibratory and position senses were intact. Audiometry studies were not done, but the patient reported no hearing changes. The neurological diagnosis was a peripheral and autonomic neuropathy secondary to cisplatin. A cbc, SMA-18, T_3-T_4, and serum renin levels were within normal ranges. A computerized axial tomographic scan of the brain was negative. A cerebrospinal fluid examination was not performed. Vigorous hydration with normal saline was initiated, but only minimal subjective and objective improvements were noted. The patient's blood pressure did not improve with the use of salt tablets or fluorocortisone over a 2-week period. Following the administration of dextroamphetamine (10 mg orally daily) and the discontinuation of the cisplatin from the combination chemotherapy, the blood pressure returned to 110/70 supine and 100/70 erect with a pulse rate of 88/minute in both positions within 24 hours. The symptoms and signs of the combined neuropathy improved somewhat over the following weeks.

DISCUSSION

Cisplatin is a cell-cycle nonspecific cytotoxic agent that has activity in the treatment of a variety of neoplasms, most notably testicular carcinoma (1). It has been studied for the treatment of bronchogenic carcinoma in combination with cyclophosphamide and doxorubicin (2). Cisplatin has substantial gastrointestinal and nephrotoxic effects. It has also been described as a neurotoxin causing ototoxicity and, less frequently, vestibulopathy and peripheral neuropathy (3-5). It has not been described in association with an autonomic neuropathy.

Peripheral neuropathy has been well-described in association with bronchogenic carcinoma (6). It is felt to represent a paraneoplastic phenomenon usually unrelated to tumor stage. The etiology of the neuropathy is unknown, although it has been attributed to altered host immunity, infection, or tumor-secreting toxins (7).

In our patient, no signs of peripheral or autonomic neuropathy were evident prior to the chemotherapy. The patient's nutritional status was considered excellent. Serum folate and B_{12} levels were not performed to exclude subacute combined systems disease, since the clinical setting and timing of the symptomatology did not support a diagnosis of posterolateral sclerosis.

A number of chemotherapeutic agents have been associated with neurotoxicity. Of the three agents given to this patient, only cisplatin has been associated with neurological disease. Cyclophosphamide has been associated with sensations of warmth and dizziness when given as a rapid iv bolus, but these side effects usually abate within minutes (8). No peripheral or autonomic neuropathy has ever been reported in association with cyclophosphamide. Doxorubicin has previously been reported to cause clinical neurotoxicity; however, several newer anthracycline derivatives (aclarubicin) appear to have neurotoxic potential (4). CAP has not been previously reported to cause an autonomic neuropathy.

Cisplatin is potentially neurotoxic, with side effects including ototoxicity, retrobulbar neuritis, and peripheral neuropathy. Most patients who develop a cisplatin-induced neuropathy do so after having received a cumulative dose of 210-825 mg/m^2, usually corresponding to three or four courses of therapy (9). Symptoms were usually reversible in most instances, but occasional long-term deficits occurred which were exacerbated by additional cisplatin therapy (10). Clinical manifestations include painful paresthesias in a stocking and glove distribution, loss of vibratory sense, and decreased deep-tendon reflexes. Autonomic neuropathy has never previously been reported in association with cisplatin, and only rarely has been found with other agents (vincristine and procarbazine) (11). Treatment for autonomic neuropathy can be accomplished in many patients with systemic fluorocortisone. However, some patients remain refractory and a reasonable alternative is the use of amphetamines. Our patient was not helped by the fluorocortisone but responded dramatically to a conventional dosage of D-amphetamine.

The autonomic neuropathy described represents a rare complication of cancer chemotherapy, and cisplatin must now be included among the list of drugs known to cause this phenomenon.

REFERENCES

1. EINHORN LH, and DONOHUE J. *Cis*-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293, 1977.
2. EAGAN RT, INGLE JN, FRYTAK S, ET AL. Platinum-based polychemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer. Cancer Treat Rep 61:1339-1345, 1977.
3. VON HOFF DD, SCHILSKY R, REICHERT CM, ET AL. Toxic effects of *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 63:1527-1531, 1979.
4. KAPLAN RS, and WIERNIK PH. Neurotoxicity of antineoplastic drugs. Semin Oncol 9:103-130, 1982.
5. BRUCKNER HW, COHEN CC, DEPPE G, ET AL. Chemotherapy of gynecological tumors with platinum II. J Clin Hematol Oncol 7:619-632, 1977.
6. HALL TC. Paraneoplastic syndromes. Ann NY Acad Sci 230, 1974.
7. BLACK LF. Neoplasms of the lung. *In* Cecil Textbook of Medicine, 16th ed. W. B. Saunders Co, 1982, chap 51, p 416.
8. TASHIMA CK. Immediate cerebral symptoms during rapid intravenous administration of cyclophosphamide. Cancer Chemother Rep 59:441-442, 1975.
9. HADLEY D, and HERR HW. Peripheral neuropathy associated with *cis*-dichlorodiammineplatinum (II) treatment. Cancer 44:2026-2028, 1979.
10. KEDAR A, COHEN ME, and TREEMAN AI. Peripheral neuropathy as a complication of *cis*-dichlorodiammineplatinum(II) treatment: a case report. Cancer Treat Rep 62:819-821, 1978.
11. AISNER J, WEISS HD, CHANG P, ET AL. Orthostatic hypotension during combination chemotherapy with vincristine (NSC-67574). Cancer Chemother Rep 58:927-930, 1974.

Treatment of Patients With Metastatic Osteogenic Sarcoma: A Report From the Childrens Cancer Study Group[1,2]

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Fifty-three patients with metastatic osteogenic sarcoma were treated with vincristine, high-dose methotrexate with citrovorum factor rescue, and cisplatin. Metastases were surgically removed in most patients, either prior to chemotherapy or following initial response to therapy. Among 29 previously treated patients, responses to initial chemotherapy included two complete remissions, six partial remissions, and eight patients with stable disease. Twenty-three patients were disease-free, six for > 12 months. Toxicity was moderate, but usually reversible. There were two toxic deaths and one unexplained death 48 hours following a dose of cisplatin. [Cancer Treat Rep 68:661–664, 1984]

In patients with disseminated osteogenic sarcoma (OS), the median survival is 6 months to 1 year following demonstration of metastasis (1). When the lung is the only site of disease, pulmonary resections have resulted in 5-year survival rates of 18%–40% (1–3). Several chemotherapeutic agents are active in the treatment of OS (4–7). In 1978, the Childrens Cancer Study Group initiated a protocol to study the effectiveness of methotrexate (MTX), cisplatin (DDP), and vincristine (VCR) in the treatment of patients with metastatic OS.

MATERIALS AND METHODS

The patients were children < 21 years of age with pathologically confirmed diagnosis of recurrent and/or metastatic OS.

Chemotherapy consisted of courses of VCR (1.5 mg/m^2) and MTX (250 mg/kg) over 6 hours with citrovorum factor rescue, alternating with infusions of DDP (3 mg/kg) over 8 hours. The chemotherapy cycle was repeated every 14–21 days during the induction phase and every 6 weeks during maintenance therapy. Drugs were deferred for creatinine clearance < 60 ml/minute/1.73 m^2, absolute neutrophil count < 1000/mm^3, or platelet count < 100,000/mm^3, until the values returned to an acceptable level. Maintenance therapy began when disease-free status was attained or when stable disease was documented following at least four cycles of chemotherapy. Surgical removal of metastases was recommended either at the start of treatment or following initial chemotherapy. Patients were monitored with radiographic examination of the chest, bone scans, and computerized tomographs of the chest.

An adequate trial was defined as the completion of two cycles of chemotherapy. Patients were removed from study for progressive disease or unacceptable toxicity. All patients were evaluated for toxicity. Individual responses to the chemotherapeutic regimen in patients receiving chemotherapy prior to surgery were graded as *complete remission* (*CR*)—no evidence of tumor in any site; *partial remission* (*PR*)—a > 50% decrease in volume of all lesions; *stable disease*—nonprogression of all lesions, with no new lesions for at least 2 months; or *progressive disease*. Total disease-free interval was determined in all patients rendered disease-free.

RESULTS

Fifty-three patients (29 male, 24 female; 3–21 years of age) were eligible. Of these patients, 46 had received prior chemotherapy including MTX, VCR, and doxorubicin, and one had received a course of transfer factor. Primary tumor sites were: lower extremity, 44 patients; upper extremity, three; pelvis, four; and unknown, two. Forty-nine patients (92%) had undergone surgical ablation of the primary lesion.

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[2]Contributing Childrens Cancer Study Group investigators, institutions, and grant numbers are given in the Appendix. Public Health Service grant support (CA) is from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. *See Appendix* at end of paper.

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Only pulmonary metastases were seen in 29 patients (55%); seven had a single lesion and eight had fewer than five lesions. Skeletal and/or lymph node metastases, with or without pulmonary metastases, were seen in 24 patients (45%). More than one-half of the patients (42 of 53) had developed metastases within 12 months of diagnosis. Seventeen patients (32%), 11 of whom had received chemotherapy prior to study, had metastases at the time of diagnosis. Eleven patients (21%), four of whom were off therapy at the time, developed metastases $\geq$ 1 year after initial diagnosis (median, 19 months).

Seven patients were inevaluable because of early death (two patients) or inadequate trial (five). In six newly diagnosed patients, one had a PR and one had stable disease. In 29 previously treated patients, responses included two CRs (7%; 10+ and 8+ months), six PRs (21%), and eight with stable disease (28%), for a total response rate of 56% (16 responses among 29 patients). Limited pathological descriptions were available on metastatic lesions of eight patients following chemotherapy and showed combinations of active and inactive tumor in most patients with PR or stable disease.

Twenty-three of 46 evaluable patients (50%) were disease-free for intervals of 3-35+ months (median, 8) with either chemotherapy alone (two patients), initial surgery followed by chemotherapy (11), or chemotherapy followed by surgery (ten). Six of the patients remained disease-free for > 12 months. Of these patients, five had pulmonary lesions only and four had less than four lesions. Metastatic disease had developed within 6 months of initial diagnosis in two patients and > 1 year after diagnosis in four. The median survival of our patients was 18½ months.

Administration of this regimen produced significant toxic effects, including renal, hematologic, gastrointestinal, hepatic, neurologic, and allergic. Most of the reported toxic effects were mild or moderate and reversible. However, severe, nonfatal toxicity was reported on 46 occasions in 30 patients. The most common forms of severe toxicity were myelosuppression (Hg < 7 g/dl, absolute neutrophil count < 500/mm^3, or platelet count < 50,000/mm^3), renal dysfunction (serum creatinine > 1.5 mg/dl), and mucositis. Four of six episodes of severe renal toxicity were irreversible during the course of the study. The one incidence of cardiac toxicity consisted of left ventricular dysfunction noted on echocardiogram in a patient who had previously received doxorubicin (520 mg/m^2). Chemotherapy was discontinued in two patients due to renal and cardiac toxic effects. In addition, there were three possible treatment-related deaths. The first death was a sudden respiratory arrest 48 hours following administration of DDP. The second death was the result of renal failure secondary to MTX, with subsequent CNS catastrophe consisting of seizures and cardiorespiratory arrest. Postmortem examination revealed leukoencephalopathy. The third death resulted from pancytopenia and sepsis. Intervals between drug courses had to be increased with time in the study because of decreasing patient compliance, as well as decreasing overall tolerance for chemotherapy. Drug dosages were adjusted in a few patients with markedly decreased creatinine clearances. However, simple modification in drug scheduling or temporary omission of DDP enabled continuation of chemotherapy without development of irreversible or unacceptable toxicity in most patients.

DISCUSSION

The regimen studied is active in patients with OS who have relapsed, as demonstrated by induction of CR with chemotherapy alone in two previously treated patients and a response rate of 56% in 29 evaluable patients. Only two of six patients newly diagnosed with metastatic OS had PR or stable disease, suggesting that these patients may have more aggressive disease. We recognize that the growth rate of OS lesions may be variable and, therefore, radiologically stable disease may not represent a true response to therapy. On the other hand, stable lesions may represent disease control, since inactive lesions may persist on radiographic examinations for long periods of time. In at least three of eight patients with stable disease, surgical specimens showed no tumor or very inactive tumor.

In patients with previously treated metastatic OS, other studies have documented response rates of 33%-62% for DDP (6) and 40% for high-dose MTX-VCR with citrovorum factor rescue (7). The numbers of patients in these studies are too small for meaningful statistical comparisons. However, our response rate is comparable to the best of these and may be superior to either drug used alone.

Toxicity in our study was greater than toxicity from MTX alone, but is not significantly greater than that encountered with DDP alone. Furthermore, toxicity was manageable and usually reversible with appropriate modifications of drug schedules. Even the few instances of irreversible renal toxicity consisted of chemical abnormalities without clinical symptoms.

This aggressive approach of chemotherapy and surgery has resulted in significant disease-free intervals in approximately one-half of our patients with metastatic OS. However, the major issue of the contribution of chemotherapy to the management of these patients remains unresolved. Repetitive resection of pulmonary metastases with or without additional chemotherapy has resulted in a median survival of 36 months in the Mayo Clinic review (3). That review documented the efficacy of aggressive surgery, but did not address the usefulness of chemotherapy. Our results are not directly comparable to these, since the endpoint of our study was tumor recurrence. Our estimated median survival was 18½ months. It is impossible to determine the relative contribution of

chemotherapy to those results. Perhaps large intergroup randomized trials could help evaluate the role of chemotherapy in the management of patients with recurrent OS.

APPENDIX

Principal Investigators of the Childrens Cancer Study Group

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REFERENCES

1. PEREZ C, HERSON J, KIMBALL JC, ET AL. Prognosis after metastasis in osteosarcoma. Cancer Clin Trials 1(4):315-320, 1978.
2. SPANOS PK, PAYNE WS, IVINO JC, ET AL. Pulmonary resection for metastatic osteogenic sarcoma. J Bone Joint Surg 58A:624-628, 1976.
3. HAN MT, TELANDER RL, PAIROLERO PC, ET AL. Aggressive thoracotomy for pulmonary metastatic osteogenic sarcoma in children and young adolescents. J Pediatr Surg 16:928-933, 1981.
4. MOSENDE C, GUTIERREZ M, CAPARROS B, ET AL. Combination chemotherapy with bleomycin, cyclophosphamide and dactinomycin for the treatment of osteogenic sarcoma. Cancer 40:2779-2786, 1977.
5. CORTES EP, HOLLAND JF, WANG JJ, ET AL. Adriamycin (NSC-123127) in 87 patients with osteosarcoma. Cancer Chemother Rep Part 3, vol 6(2):305-313, 1975.
6. BAUM ES, GAYNON P, GREENBURG L, ET AL. Phase II study of *cis*-dichlorodiammineplatinum(II) in childhood osteosarcoma: Children's Cancer Study Group Report. Cancer Treat Rep 63:1621-1627, 1979.
7. PRATT CB, HOWARTH C, RANSOM JL, ET AL. High-dose methotrexate used alone and in combination for measurable primary or metastatic osteosarcoma. Cancer Treat Rep 64:11-20, 1980.

Evaluation of Combined Cyclophosphamide and Methotrexate Therapy in the Treatment of Metastatic Carcinoid Tumor and the Malignant Carcinoid Syndrome[1]

Charles G. Moertel,* Michael J. O'Connell, Richard J. Reitemeier, and Joseph Rubin[2]

Sixteen patients with metastatic carcinoid tumor and the malignant carcinoid syndrome were treated with combined cyclophosphamide and methotrexate therapy in a regimen previously described as highly effective. Toxicity was relatively mild and consisted primarily of leukopenia. One patient experienced some symptomatic benefit and minor reduction in hepatomegaly and 5-hydroxyindoleacetic acid excretion. None met our criteria for an objective response. Combined cyclophosphamide and methotrexate therapy does not appear to offer significant benefit for this disease. [Cancer Treat Rep 68:665–667, 1984]

Metastatic carcinoid tumor presents a fascinating challenge for oncologic management. The disease tends to be very indolent in progression, with patients frequently living several years and occasionally many years after distant metastasis has been proven. Management is made more complex by the frequent occurrence of the malignant carcinoid syndrome. Because of the rarity of this disease, chemotherapy experience has been limited and usually reports are based on very small case numbers. Responsiveness has been claimed to a variety of treatment regimens including 5-FU and doxorubicin used as single agents as well as combinations of streptozocin with either 5-FU or cyclophosphamide (1,2). The most favorable experience in the literature has been that of Mengel (3), who reported six objective responses among 11 patients treated with a combination of methotrexate and cyclophosphamide. Because this report appears in one of the major oncology textbooks, this combination could perhaps be regarded by many oncologists as standard treatment for this disease. Since no confirmation of this favorable experience has been documented, we felt that such a study was appropriate.

MATERIALS AND METHODS

All patients accepted for study were required to have histologic confirmation of metastatic carcinoid tumor. In addition, they were required to have one or more indicators of objective response to therapy. These could include a mass lesion measurable in two dimensions or malignant hepatomegaly, provided the liver was proven to contain metastatic cancer and extended at least 5 cm below the costal margin or the xyphoid process on quiet respiration. Perfusion defects on radioactive liver scan were accepted if they were clearly defined and measured at least 5 cm in greatest diameter. In the absence of measurable tumor an elevated urinary excretion of 5-hydroxyindoleacetic acid (5HIAA) was accepted, provided the level was at least 25 mg in 24 hours (normal to 6 mg). Patients were excluded from protocol entry if they were totally disabled, were unable to maintain reasonable oral nutrition (< 1200 calories/day), had coexistent malignant disease or preexistent malignant disease within 3 years prior to entry, had a leukocyte count < 4000/mm^3 or a platelet count < 130,000/mm^3, or had had any prior therapy with methotrexate or cyclophosphamide or with any other alkylating agent. Entry was deferred for at least 1 month after any prior major surgery, chemotherapy, or radiation therapy.

Cyclophosphamide was given by a single iv injection of 1000 mg/m^2 and repeated every 5 weeks. Methotrexate was given by im injection at a dose of 20 mg/m^2 on Days 1, 8, 15, and 22 of each treatment cycle. To avoid precipitating a carcinoid crisis patients who had a florid clinical carcinoid syndrome or who had urine 5HIAA excretion > 150 mg/24 hours were given 50% of the planned dose over the first cycle of treatment. If this was well-tolerated, chemotherapy was raised to full dose on the second treatment cycle. Provision was made for dosage reduction in the event that severe toxicity was encountered,

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but these reductions were, in fact, never required. Treatment was continued until progressive disease was documented.

Patients were evaluated for response by serial 5HIAA determinations as well as by complete medical examinations including measurements of indicator lesions conducted before the initiation of each 5-week cycle. Objective responses were declared for mass lesions if there was a 50% reduction in the product of the longest perpendicular diameters and for malignant hepatomegaly if the sum of liver measurements below each costal margin at the midclavicular lines and the xyphoid process had decreased by at least 30%. If urine 5HIAA excretion alone was employed as an indicator of response it was required that the 24-hour excretion be reduced by at least 50% in comparison to pretreatment levels. Objective progression was declared if there was a 25% increase in mass lesions, malignant hepatomegaly, or urine 5HIAA or if new areas of malignant disease developed.

RESULTS

A total of 16 patients were entered in the study, all of whom were eligible and included in analysis. The characteristics of these patients are shown in table 1. The seven patients with prior chemotherapy had primarily received regimens involving 5-FU, doxorubicin, and streptozocin. All patients had elevated urinary 5HIAA excretions. The median value was 213 mg in 24 hours (range, 11–599). Fifteen patients had symptoms of the malignant carcinoid syndrome (flushing, 12 patients; diarrhea, 14). The single patient without a symptomatic syndrome had a 380-mg 5HIAA excretion in 24 hours.

Therapeutic Results

None of the patients experienced an objective response according to the criteria of this study. Twelve patients had stable disease, for a median duration of 5 weeks (range, 5–25). One patient died at 10 days after the start of therapy, of an apparently unassociated medical problem (severe coronary insufficiency). Only one patient showed perceptible improvement that, while not meeting our criteria for objective response, did seem to be a definite favorable effect of drug treatment maintained for a period of 4 months. This patient had a 24% reduction in liver size as well as a reduction in urine 5HIAA excretion from 517 to 260 mg/24 hours. Diarrhea was substantially improved, whereas flushing remained unchanged. No other patient had substantive reduction in urinary 5HIAA levels. The median interval to disease progression in this group of patients was 10 weeks, with a range to 35 weeks. Twelve patients have died, while four remain alive between 3 and 24 months after the start of therapy. The estimated median survival is 6 months.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Male/female	12/4
Median age in yrs (range): 57 (36–76)	
Primary tumor	
Small bowel (ileum)	11
Lung	1
Unknown	4
Sites of metastases	
Liver	15
Peritoneal	2
Lung	1
Bone	1
No prior radiation therapy or chemotherapy	9
Performance status *	
0	3
1	7
2	2
3	4

* Eastern Cooperative Oncology Group criteria: 0 (fully active) to 4 (totally disabled).

Toxicity

Twelve patients were treated with the full planned chemotherapy dosage either initially or after escalation when a 50% dose was well-tolerated. Four patients were treated only with reduced dosages because of early death (one patient), significant leukopenia at the reduced dose (one), or exacerbation of the carcinoid syndrome at the reduced dosage (two).

The most common toxic reaction observed was leukopenia, which was seen in 80% of the patients treated with the full dose. The median wbc count nadir among these patients was 2700/mm^3, with a range of 1900–3300. Nausea, vomiting, stomatitis, alopecia, and thrombocytopenia were less commonly observed and were never severe. Two patients had severe but transient exacerbations of the carcinoid syndrome after full-dose therapy, one with greatly intensified flushing and one with diarrhea reaching 30 stools in 24 hours.

DISCUSSION

Clearly, the results of this study were negative, with only one patient showing any degree of improvement and none of the 16 patients meeting the criteria established for a partial objective response. The reliability of clinical evaluation of these patients is confirmed by the fact that only one patient had substantive reduction in 5HIAA excretion, but even this did not reach 50%. Our failure to observe any meaningful evidence of antineoplastic activity is evidenced by the median interval to disease progression of only 10 weeks, a very short time for such a generally slow-moving malignant disease. The striking

difference between our results and the previously reported 55% objective response rate with this drug combination is difficult to explain. In view of the usually indolent course of metastatic carcinoid tumor it is our policy to defer chemotherapy until patients have significant symptoms related to their disease. Our patients, therefore, had considerable tumor bulk. Nevertheless, they were generally in very good condition, with most being capable of working full- or part-time and most having no prior chemotherapy or radiation therapy exposure. With the same patient selection and criteria for response, our institution has obtained a 21% response rate among 33 patients treated with doxorubicin, 26% among 19 patients treated with 5-FU, and 33% among 43 patients treated with combined 5-FU and streptozocin.

We reviewed our study with Dr. Mengel (personal communication, 1983) seeking possible reasons for our disparate results. In his pioneering work with this combination he had explored a variety of specific dosage schedules, but he felt that our regimen was fully representative of these. Essentially, all of his patients had had no prior chemotherapy. Paradoxically, his patients had substantially more disability than ours, with most requiring hospitalization. Also, they probably had a greater tumor bulk, since the median 5HIAA levels were between 300 and 400 mg/24 hours. He felt that his responders were primarily those with very aggressive disease.

Based on our data we must conclude that the combination of cyclophosphamide and methotrexate as employed in this study and in our study population offered no important treatment benefit for metastatic carcinoid tumor and cannot be recommended as standard therapy for this disease.

REFERENCES

1. DAVIS Z, MOERTEL CG, and MCILRATH DC. The malignant carcinoid syndrome. Surg Gynecol Obstet 137:627–644, 1973.
2. MOERTEL CG, and HANLEY JA. Combination chemotherapy trial in metastatic carcinoid tumor and the malignant carcinoid syndrome. Cancer Clin Trials 2:327–344, 1979.
3. MENGEL CE. The carcinoid syndrome. *In* Cancer Medicine (Holland JF, and Frei E, eds). Philadelphia, Lea & Febiger, 1982, pp 1818–1826.

Activity of Mitolactol in Cancer of the Uterine Cervix[1,2]

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Antitumor activity has been documented in this pilot study utilizing mitolactol in patients with advanced carcinoma of the cervix. These results may in part be explained by optimal patient selection; however, the results do encourage further testing of this hexitol in this disease. [Cancer Treat Rep 68:669–670, 1984]

Between October 1980 and June 1982, patients with advanced (stage IV) or recurrent squamous cell cancer of the uterine cervix were entered in a protocol study evaluating the activity of two single drugs: mitolactol (DBD) or cisplatin. The study represented a collaborative effort of the Hospital de Oncologia (National Medical Center, Mexico) and the New York University (NYU) Medical Center. Patient eligibility, which was similar in both institutions, included normal renal and hematologic functions (wbc counts $\geq 4000/mm^3$ and platelet counts $\geq 125{,}000/mm^3$), presence of measurable parameters of disease, and Eastern Cooperative Oncology Group (ECOG) performance status of 2 or better. At least 4 weeks had to elapse from prior irradiation, and at least 3 months if irradiation had been directed to the pelvis. In Mexico, all patients allocated to receive DBD ($180 mg/m^2$) for 10 days every 4 weeks were previously untreated with chemotherapy. Assignment to either DBD or cisplatin was made according to availability of inpatient beds (cisplatin) or desirability of total outpatient treatment (DBD). At NYU, DBD was given only after failure of cisplatin. The current report describes only the experience with DBD; the cisplatin arm, which included a comparison of two dose schedules, is still ongoing. The study made no attempt to compare the two drugs; rather, it was designed as a simultaneous phase II evaluation of two drugs. Standard ECOG criteria of response for complete regression, partial regression, and no response were used.

RESULTS

The age, indicator lesion, and response of the 18 patients entered from Mexico are shown in table 1. Toxic manifestations were remarkably mild, with only one patient with grade 2 leukopenia (ECOG criteria) and five with grade 1. Grade 1 leukopenia was persistent in the instance of one patient (No. 17) who received four cycles. Two patients experienced grade 1 thrombocytopenia. Gastrointestinal toxicity was absent. At NYU, four patients were entered but only three completed one cycle of treatment. No response was achieved in these heavily pretreated patients with pelvic recurrences.

Analysis of these results indicates an overall objective response rate of 28% (five responses among 18 patients) among untreated patients with carcinoma of the cervix in Mexico; if all evaluable patients were considered, including the evaluable patients from NYU, the overall response rate is 24% (five responses among 21 patients). The median duration of response was 6 months (range, 6–11) and the median survival of responders and nonresponders was 12+ and 7.5 months, respectively. It is noteworthy that three of five patients with pulmonary metastases responded, whereas only two of 12 with pelvic disease had objective responses. The most remarkable response was achieved in a 40-year-old woman manifesting lung metastasis 2 years after treatment with irradiation for stage IIB cancer. The lung lesions disappeared after the second cycle, only to return 11 months after start of DBD treatment. The patient has subsequently shown a partial response to cisplatin and remains alive 22+ months after beginning chemotherapy.

DISCUSSION

Initial studies of dianhydrogalactitol by the Gynecologic Oncology Group (GOG) documented some anti-

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TABLE 1.—Analysis of responses to DBD*·† (Mexican patients)

Patient No.	Age (yrs)	Site of indicator lesions	Duration of response (mos)‡	Toxicity and status
1	40	Lungs	CR (11)	Alive 22+ mos on cisplatin
2	53	Pelvis	PR (6)	Died 14 mos, grade 1 leukopenia
3	57	Lungs	PR (6)	Alive 11+ mos, grade 1 leukopenia and thrombocytopenia on cisplatin
4	60	Pelvis	PR (6)	Alive 11+ mos, grade 1 leukopenia on cisplatin
5	49	Lungs	PR (8+)	Alive 8+ mos
6	49	Pelvis	NR	Died 4 mos
7	34	Pelvis	NR	Died 3 mos
8	37	Pelvis	NR	Died 4 mos, grade 2 leukopenia
9	56	Pelvis, lungs	NR	Died 8 mos, grade 1 leukopenia and thrombocytopenia
10	50	Pelvis	NR	Died 7 mos
11	65	Lung	NR	Died 3 mos
12	42	Pelvis	NR	Died 8 mos, grade 1 leukopenia
13	56	Pelvis	NR	Died 13 mos
14	50	Pelvis	NR	Died 13 mos
15	41	Pelvis	NR	Died 7 mos
16	54	Pelvis, neck node	NR	Died 14 mos
17	40	Pelvis	Stable disease (4)	Died 15 mos, grade 1 leukopenia, persistent
18	61	Pelvis	Stable disease (5)	Alive 15+ mos, grade 1 leukopenia on cisplatin

* Initial performance status (ECOG scale): all 0 or 1.
†Prior radiotherapy to pelvis: all patients > 1 yr prior to entry. No patient received additional palliative radiotherapy.
‡CR = complete response; PR = partial response; NR = no response.

tumor activity (1). The drug was disappointing in combination with cisplatin (2), and additional clinical trials have focused on other hexitols. With the current experience, we conclude that DBD also has activity against cervical cancer worth pursuing further. Accordingly, we have begun a trial with the drug given at a dose of 240 mg/m^2 for 10 days every 28 days. This study, which is being run in collaboration with other ECOG institutions, will extend current observations at dosages expected to produce greater hematologic toxicity. If these observations are confirmed, the drug might prove to be suitable for combination trials.

This study also indicates that patient selection may affect duration of response and survival. Such selection explains why our nonresponders lived more than twice as long as the nonresponders in a recent trial of cisplatin by the GOG (3). Conversely, responses to combinations by US groups have not been very durable (4). Patients with lung metastases have easily measurable lesions and long responses, as noted by investigators in three different countries (5–7). This suggests such patients may be most suitable for comparative trials.

REFERENCES

1. BLOM J, BLESSING J, MLADINEO J, ET AL. Dianhydrogalactitol (DAG) in the treatment of advanced gynecologic malignancies. Proc Am Assoc Cancer Res and ASCO 21:416, 1980.
2. VOGL SE, SELTZER V, CAMACHO F, ET AL. Dianhydrogalactitol and cisplatin in combination for advanced cancer of the uterine cervix. Cancer Treat Rep 66:1809–1812, 1982.
3. BONOMI P, BRUCKNER H, COHEN C, ET AL. A randomized trial of three cisplatin regimens in squamous cell carcinoma of the cervix. Proc ASCO 1:110, 1982.
4. BAKER LH, OPIPARI MI, and IZBICKI RM. Phase II study of mitomycin C, vincristine and bleomycin in advanced squamous cell carcinoma of the uterine cervix. Cancer 38:2222–2224, 1976.
5. LIRA-PUERTO VM, HIDALGO IN, MORALES FR, ET AL. Bleomycin, methotrexate and cyclophosphamide in advanced squamous cell carcinoma of the uterine cervix. Proc Am Assoc Cancer Res and ASCO 20:319, 1979.
6. SAIGO N. Chemotherapy against pulmonary metastasis from uterine cervical carcinoma. Gan to Kagakuryoho 9:992–997, 1982.
7. ALBERTS DS, and SURWIT EA. Mitomycin C, bleomycin, vincristine and cisplatin combination therapy for advanced, recurrent cervix cancer: a Southwest Oncology Group pilot study. *In* Mitomycin C: Current Impact on Cancer Chemotherapy (Ogawa M, Rozencweig M, and Staquet MJ, eds). Excerpta Medica, 1982, pp 127–134.

Chemotherapy for Advanced Adenocarcinoma and Squamous Cell Carcinoma of the Lung With Etoposide and Cisplatin[1,2]

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Forty-one patients with unselected advanced non-small cell lung cancer were treated with a combination of etoposide and cisplatin. A response rate of 19%, a 78-week median survival of responders, and a 36-week overall median survival were observed. [Cancer Treat Rep 68:671–673, 1984]

Cancer of the lung is the most lethal cancer in the United States. It is expected to account for approximately 135,000 new cases and 117,000 deaths in 1983 (1). Current therapy is so ineffective that only 10% of all patients diagnosed have been observed to live > 5 years (2). Most patients (80%) have advanced-stage lung cancer and are inoperable at presentation; median survival for such patients is 30 weeks for limited disease and 13 weeks for extensive disease (3). The quest for new drugs and combinations of drugs effective in lung cancer continues. The activity of cisplatin (CDDP) (4–6) and the epipodophyllotoxin derivative etoposide (VP-16) (7) has been well-documented in lung cancer of adenocarcinoma and squamous cell carcinoma histologies. However, these responses are of short duration and do not substantially influence patient survival (4–8). Observation of synergy between VP-16 and CDDP in murine tumors (9) prompted studies of this combination in non-small cell lung cancer (10,11) and in small cell lung cancer (12). A response rate of 47% was observed in squamous cell lung cancer in the European Organization for Research on Treatment of Cancer (EORTC) study (10) and of 39% in non-small cell lung cancer in another study (11). Our study was undertaken to confirm the findings of the European studies and to further define the effects of this treatment regimen in advanced adeno-squamous cell lung cancer (ASCLC).

MATERIALS AND METHODS

From February 1981 to June 1981, 41 patients with ASCLC who had measurable disease were registered for combination chemotherapy with VP-16 and CDDP. None of the patients had previously received chemotherapy. If patients had previously been treated with irradiation or surgery, they were included after documented relapse. All patients except three had tumor spread beyond the confines of one hemithorax. There were no age or performance status limitations. A pretreatment creatinine clearance of > 60 ml/minute and adequate bone marrow function as evidenced by peripheral hemogram were required. Patients were required to understand the experimental nature of the treatment and to sign the consent form approved by the Surveillance Committee of the M. D. Anderson Hospital and Tumor Institute prior to entry in this study.

Patients were evaluated with complete history and physical examination, relevant laboratory tests, and radiologic and nuclear scan studies. Sputum examination and bronchoscopic or needle aspiration biopsy were used where appropriate for histologic diagnosis. Patients were followed with measurement of objective tumor parameters before each course of treatment using appropriate studies.

On the first day of chemotherapy, patients received CDDP at a dose of 60 mg/m^2 of body surface area over 3–4 hours, with prehydration and administration of 15 g of mannitol before and after CDDP. On the fourth, sixth, and eighth day of chemotherapy, VP-16 was given in doses of 120 mg/m^2 of the body surface area in 1 L of normal saline over 4 hours. Each treatment cycle was repeated every 3–4 weeks upon recovery from myelosup-

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pression and other toxic effects. The doses of CDDP and VP-16 were increased or decreased with an attempt to achieve a nadir granulocyte count between 750 and 1000/mm^3 and nadir platelet count between 75,000 and 100,000/mm^3. CDDP was discontinued if creatinine clearance dropped to < 60 ml/minute or serum creatinine increased to > 2.0 mg/dl.

Standard criteria were used for response assessment, as previously published (13). Statistical comparisons were made using the chi-square test with Yate's correction. Patient survival, response duration, and their significance were evaluated by the method of Kaplan and Meier (14) and the generalized Wilcoxon test (15). The response duration was measured from the date response was first documented and the survival was measured from the start of treatment until progression of disease and death, respectively.

RESULTS

Four patients were considered inevaluable. One early death on Day 12 after chemotherapy occurred during pericardiocentesis, and the causes for discontinuation of treatment during or after the first course in three patients were VP-16 anaphylaxis, iliofemoral thrombosis, and rapid deterioration of performance status. Characteristics of all patients are given in table 1. A preponderance of adenocarcinoma (61%) is notable. Central nervous system metastasis was present at the time of diagnosis in three (7%) patients. Ten (24%) patients had previously received radiation therapy to the primary tumor; they presented with distant relapse in eight patients and local relapse in two.

Nineteen percent of the evaluable patients achieved complete response or partial response (responders). If three patients with limited disease are excluded, the response rate is 18% (six responses among 34 patients). Fifty-four percent of patients experienced disease stabilization and 27% had progression of their tumor. Response rates were evaluated for patient characteristics including histology, performance status, and pretreatment weight loss. Although the response rate for adenocarcinoma (13%) was less than that for squamous cell carcinoma (25%) and large cell carcinoma (33%), the difference is not statistically significant ($P = 0.47$). Patients with a performance status of 2 or better had a slightly better response rate, but the difference was also statistically insignificant ($P = 0.57$). Pretreatment weight loss appeared to have no significant impact on response in this group of patients. The duration of response in responding patients was 26 weeks. The median survival of responders was 78 weeks, while the median survival of all patients was 36 weeks. The improved survival of responders is significant ($P = 0.00005$). One limited- and one extensive-disease patient achieved complete response and are alive at 95 and 89 weeks, respectively, since starting treatment, with progression of disease in the latter patient.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total	41
Median age in yrs (range): 56 (35-76)	
Sex: male/female	35/6
Histology	
Adenocarcinoma	25 (61)
Squamous cell carcinoma	9 (22)
Large cell carcinoma	7 (17)
Pretreatment weight loss	
< 6%	21 (51)
≥ 6%	19 (46)
Unknown	1 (2)
Performance status (Zubrod scale)	
0-2	32 (78)
3,4	9 (22)

No chemotherapy-related deaths occurred. Myelotoxicity was moderate. A granulocyte count nadir of ≤ 1000/mm^3 occurred in 33% of evaluable courses and a platelet count nadir of ≤100,000/mm^3 occurred in 23% of evaluable courses. There were seven episodes of fever of unknown origin and nine documented infections. One episode of life-threatening pseudomonas septicemia was documented in a severely debilitated patient. Nausea and/or vomiting were almost universal (93% of courses). Azotemia, defined as elevation of serum creatinine to > 1.4 mg/dl, occurred in five (13%) patients and was transient in three. CDDP had to be discontinued in one patient due to progressive increase in serum creatinine and decrease in creatinine clearance after six courses of chemotherapy. Minor neuropathy occurred in one patient. Other toxic effects included alopecia, anorexia, and diarrhea.

DISCUSSION

The response rate of 19% in this study is comparable to that in the European studies using the combination of CDDP and VP-16 in ASCLC, when prognostic indicators are taken into account. Response rates of 35% and 39% were reported by these studies (10,11). A review of prognostic indicators in the study of Goldhirsch et al (11) and an update of the EORTC study (16) reveal that they included 44% and 43% of patients with limited disease, and 61% and 75% of patients with squamous cell carcinoma, respectively. The performance status of all the patients was 2 or better in the EORTC study, and 3 or better in the study by Goldhirsch. No information about pretreatment weight loss is included in either of these studies. The present study is significantly different in its preponderance of adenocarcinoma (61%), more advanced disease (93% of patients with extensive disease), and in-

clusion of patients with performance status of 3 and 4 (22%).

The observed median survival of 78 weeks for responders and 36 weeks for all patients in this study compares favorably with the 60 and 23 weeks, respectively, observed in the EORTC study (16) and is encouraging. Similarly prolonged survival (94 weeks) was reported by Gralla et al (8) using vindesine and CDDP, who also observed that responders receiving high-dose CDDP in combination with vindesine had significantly longer survival in comparison to low-dose regimens, although the response rates in the two treatment arms were similar (40% vs 46%). The improved overall survival and survival of responders are encouraging and deserve further exploration. Our trial comparing the VP-16 plus CDDP regimen with the vindesine and high-dose CDDP combination has been completed and will be reported in the near future. Low response rates in patients with ASCLC who are in poor prognostic groups underscore the need for new and more effective chemotherapy agents.

REFERENCES

1. SILVERBERG E. Cancer statistics 1983. CA 33:9-25, 1983.
2. SILVERMAN DT. Cancers of the respiratory system. *In* Cancer Patient Survival. Report 5, US Department of Health, Education, and Welfare, 1976, pp 136-156.
3. STANLEY KE. Prognostic factors for survival in patients with inoperable lung caner. JNCI 65:25-32, 1980.
4. DE JAGER R, LONGEVAL E, and KLASTERSKY J. High-dose cisplatin with fluid and mannitol-induced diuresis in advanced lung cancer: a phase II clinical trial of the EORTC Lung Cancer Working Party (Belgium). Cancer Treat Rep 64:1341-1346, 1980.
5. PENETTIERE FJ. Effects of the two dose SWOG *cis*-platinum program in non-oat cell lung cancer. Proc Am Assoc Cancer Res and ASCO 21:363, 1980.
6. BERENZWEIG M, VOGL SE, KAPLAN BH, ET AL. Phase II trials of *cis*-diamminedichloroplatinum in patients with non-small cell bronchogenic carcinoma not exposed to prior chemotherapy. Proc Am Assoc Cancer Res and ASCO 21:457, 1980.
7. CAVALLI F. VP16-213 (etoposide): a critical review of its activity. Cancer Chemother Pharmacol 7:81, 1982.
8. GRALLA RJ, CASPER ES, KELSEN DP, ET AL. Cisplatin and vindesine combination chemotherapy for advanced carcinoma of the lung: a randomized trial investigating two dose schedules. Ann Intern Med 5:414-420, 1981.
9. MABEL JA, and LITTLE AD. Therapeutic synergism in murine tumors for combination of *cis*-dichlorodiammeplatinum with VP16-213 or BCNU. Proc Am Assoc Cancer Res and ASCO 20:230, 1979.
10. LONGEVAL E, DE JAGER R, TAGNON H, ET AL. *Cis*-platinum (CDDP)-VP16-213 combination chemotherapy in non-small cell (NSC) bronchogenic cacinoma: phase I-II clinical trial. Proc Am Assoc Cancer Res and ASCO 21:368, 1980.
11. GOLDHIRSCH A, JOSS RA, CAVALLI F, ET AL. *cis*-Dichlorodiammineplatinum (II) and VP16-213 combination chemotherapy for non-small cell lung cancer. Med Pediatr Oncol 9:205-208, 1981.
12. SIEROCKI JS, HILARIS BS, HOPFAN S, ET AL. *cis*-Dichlorodiammineplatinum(II) and VP16-213: an active induction regimen for small cell carcinoma of the lung. Cancer Treat Rep 63:1593-1597, 1979.
13. VALDIVIESO M, BODEY GP, BENJAMIN RS, ET AL. Role of intravenous hyperalimentation as an adjunct to intensive chemotherapy for small cell bronchogenic carcinoma. Cancer Treat Rep 65(Suppl 5):145-150, 1981.
14. KAPLAN EL, and MEIER P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.
15. GEHAN EA. A generalized Wilcoxon's test for comparing arbitrarily censored samples. Biometrika 52:203-223, 1958.
16. KLASTERSKY J, LONGEVAL E, NICAISE C, ET AL. Etoposide and *cis*-platinum in non-small cell bronchogenic carcinoma. Cancer Treat Rev 9(Suppl A):133-138, 1982.

Phase II Study of Weekly Low-Dose 4′-Epi-doxorubicin in Advanced Postmenopausal Breast Cancer[1]

William G. Jones[2,*] and Willy Mattsson[3]

A phase II study of 4′-epi-doxorubicin in 39 fully evaluable patients with advanced postmenopausal breast cancer has been performed. The patients received 20 mg of 4′-epi-doxorubicin iv weekly, irrespective of surface area. This dose schedule follows previous work with doxorubicin, showing good therapeutic effect with very low toxicity. The preliminary results of this study of 4′-epi-doxorubicin confirm that the drug is very active in breast cancer (51% objective response rate) and that the low-dose weekly approach is well-accepted with remarkably low toxicity (59% of patients had no complaints of toxic effects). [Cancer Treat Rep 68:675–677, 1984]

4′-Epi-doxorubicin is one of a series of compounds derived from doxorubicin. A recent review by Ganzina (1) summarizes the information to date on preclinical and clinical studies and suggests that this compound is active in breast cancer, is at least as effective as the parent drug, and would appear to be less toxic.

For some time, doxorubicin has been regarded as the most effective cytotoxic drug in advanced breast cancer (2), but its toxicity prompted Mattsson et al (3) to adopt a low-dose weekly schedule. This has proved effective in lowering toxicity without loss of potency. With this background, a phase II study was initiated with low-dose weekly 4′-epi-doxorubicin.

MATERIALS AND METHODS

Patients of any age with advanced postmenopausal breast cancer resistant to or unlikely to respond to conventional therapy were entered in this study. Histological proof of the primary disease was required and only patients with two-dimensionally measurable soft tissue or visceral metastases were included. Bone metastases were not accepted for measurement of objective response. Previous therapy with cytotoxic agents did not disqualify patients if they had normalization of blood cell count. Prior or concomitant radiotherapy was acceptable, provided it did not interfere with the assessment of the marker lesion(s). An initial wbc count of 2×10^9/L and a platelet count of 100×10^9/L were required. Patients with another type of neoplasm, or who had other medical conditions or circumstances which possibly precluded adherence to the treatment or assessment schedule, were excluded. Informed consent was obtained from patients entering the study. 4′-Epi-doxorubicin was given either by bolus iv injection or injection into the tubing of a fast-running iv drip. A dose of 20 mg/week (irrespective of the patient's surface area) was decided upon empirically for simplicity.

The World Health Organization (WHO) criteria of response (4) were used. Measurable lesions were assessed before treatment and then every 4 weeks, with treatment continuing only if a response or a no change category existed. If the patient continued to respond at the 12th week, the frequency between injections could be decreased to 2–3 weeks at the discretion of the investigator.

Toxic effects were assessed from the start of treatment. Before each weekly dose, a cbc and routine biochemistry, including tests of liver function, were undertaken. A chest x-ray and ECG were performed every 4 weeks, at the time of assessment of response. ^{99m}Tc bone scan, computerized tomographic scan of liver/abdominal cavity, and x-rays of skeleton were performed when clinically indicated and were followed if initially pathological. The patients were questioned about toxic effects each week. Antiemetics or other drugs were not routinely given to prevent toxic effects.

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TABLE 1.—Pretreatment patient characteristics*

	Karlstad	Leeds	Total
Entered	19	24	43
Evaluable	18	21	39
Age			
Median	65	64	65
Range	42-84	42-89	42-89
Sex			
Male	0	1	1
Female	19	23	42
WHO performance status†			
Mean	2	2	2
Range	1-3	0-3	0-3
Dominant metastases‡			
Soft tissue	2	13	15
Bone§	4	2	6
Viscera	12	6	18
Prior therapy for metastases‡	16	18	34
None	2	3	5
Tamoxifen	16	16	32
High-dose medroxyprogesterone	6	0	6
Estrogen	0	5	5
Castration	4	1	5
Other hormone	4	1	5
VAC/VACM chemotherapy¶	7	1	8
Other chemotherapy**	11	1	12

*Unless otherwise specified, values = No. of patients.
†*See* ref 4.
‡Evaluable patients only.
§Patients with mainly bone lesions also had measurable soft tissue/visceral lesions.
¶V = vincristine; A = doxorubicin; C = cyclophosphamide; and M = methotrexate.
**Excluding doxorubicin.

RESULTS

Forty-three patients were entered in the study between March 1982 and May 1983. Thirty-nine patients were fully evaluable. The pretreatment characteristics by institution are shown in table 1. Thirty-four patients had received at least one type of therapy for their metastases. None of the patients had received adjuvant chemotherapy. The performance status was poor (median, 2). When a response occurred, the performance status was improved in all cases.

An objective response rate (complete response [CR] + partial response [PR]) of 51% was observed (table 2). An objective response could be assessed at 4 weeks but, in one case, a no change category changed to a PR at Week 11 and, occasionally, response was seen clinically to be slow. Follow-up is still short, but it seems that the mean duration of response is related to the response category (table 2). Ten patients continue to be in the study.

The treatment was extremely well tolerated. The majority of toxic effects were very slight and did not occur with every treatment cycle; some were probably not treatment-related. Some patients complained of more than one type of effect. Routine preventive measures,

TABLE 2.—Response to treatment of 39 patients

Response*	No. of patients (%)	Duration of response (wks)†	
		Range	Mean
CR	2 (5)	48+ to 52	50+
PR	18 (46)	4 to 56	30+
No change	11 (28)	4 to 40+	14+
Disease progression	8 (21)	—	—

*CR + PR = 20 of 39 patients (51%).
†Plus sign indicates continuing response.

TABLE 3.—Toxic effects of treatment

	No. of patients (%)
Total	39 (100)
Toxic effects	
Nausea only	8 (21)
Slight alopecia	8 (21)
Phlebitis	4 (10)
Nausea and vomiting	3 (8)
Sore mouth	2 (5)
Dizziness	2 (5)
Lethargy	1 (3)
Diarrhea	1 (3)
Mental depression	1 (3)
Dry skin	1 (3)
Hematological	0 (0)
Cardiac	0 (0)
Liver	0 (0)
No toxic effects reported	23 (59)

such as antiemetics, were not employed unless the symptom/effect was persistent over a number of courses. All possible effects were recorded, the majority were negligible, and 23 (59%) patients had no complaints (table 3).

In 269 courses of treatment, only once was a wbc count $< 2.5 \times 10^9$/L observed (2.3×10^9/L). The mean wbc count was 5.9×10^9/L. The lowest platelet count recorded was 135×10^9/L, and the mean count was 275×10^9/L. On no occasion was treatment withheld because of a low count. No clinical or ECG evidence of cardiac toxicity was seen. No definite conclusion can be drawn from this preliminary observation, since the maximum cumulative dose so far is only 1180 mg.

DISCUSSION

The therapy of advanced breast cancer has improved with the advent of new hormone and chemotherapeutic agents. However, the conventional use of combination chemotherapy often produces toxicity which may detract from the quality of survival while causing objective regression of disease.

The early results of this phase II study suggest that by using a low-dose weekly schedule of 4′-epi-doxorubicin, the toxicity can be reduced, while demonstrating excellent single-agent activity of this drug. An objective response could be obtained without any measurable myelosuppression, which is a new important finding in the management of solid tumors. This approach is worthy of intensive investigation, since it has potential implications in the future development of both palliative and adjuvant breast cancer chemotherapy. The mechanism(s) involved (5) in this situation of excellent anticancer effectiveness without significant toxic effects requires further investigation.

REFERENCES

1. GANZINA F. 4′Epi-doxorubicin, a new analogue of doxorubicin: a preliminary overview of preclinical and clinical data. Cancer Treat Rev 10:1-22, 1983.
2. CARTER SK. Adriamycin, a review. JNCI 35:1265, 1975.
3. MATTSSON W, BORGSTRON S, and LANDBERG T. A weekly schedule of low dose doxorubicin in treatment of advanced breast cancer. Clin Ther 5:193-203, 1982.
4. WHO Handbook for Reporting Results of Cancer Treatment. Geneva, WHO Offset Publication No. 48, WHO, pp 22-24, 1979.
5. BOSTON RC, and PHILIPS DR. Evidence of possible dose-dependent doxorubicin plasma kinetics in man. Cancer Treat Rep 67:63-69, 1983.

Phase II Trial of 4′-Epi-doxorubicin in Patients With Advanced Malignant Melanoma[1,2]

Ellin Berman,[3,*] Ephraim S. Casper[3], Jane Howard[3], and Robert E. Wittes[3,4]

Thirty-five patients with advanced malignant melanoma were treated with 4′-epi-doxorubicin iv once every 3 weeks. Most patients had not received prior chemotherapy and had good performance status. Three partial remissions and three minor regressions were observed. All patients experienced myelosuppression, but other toxic effects were minimal. 4′-Epi-doxorubicin has limited therapeutic activity in this disease. [Cancer Treat Rep 68:679–680, 1984]

Malignant melanoma remains one of the neoplasms most refractory to systemic therapy. Although responses are seen with dacarbazine, the nitrosoureas, and other drugs, used singly and in combination (1), patients with metastatic disease generally die rapidly from progressive melanoma.

Anthracyclines are active against a variety of human tumors (2). Although doxorubicin has shown no activity in patients with malignant melanoma (3), major objective responses to detorubicin have been documented (4). Newer anthracyclines with less cardiac toxicity and slightly different antitumor activity in animal systems have recently been introduced for clinical evaluation. In an early phase I study of 4′-epi-doxorubicin, one of these new analogs, objective tumor regression was seen in a patient with metastatic melanoma (5). In a phase I study of this drug at the Memorial Sloan-Kettering Cancer Center, the appropriate dose of 4′-epi-doxorubicin for use on an every 3 week schedule was determined to be 85 mg/m^2 for patients with a performance status of ≥ 70% (Karnofsky scale) or 70 mg/m^2 for patients with a lower performance status (6). The present study was designed to evaluate the effectiveness of 4′-epi-doxorubicin in the treatment of patients with advanced malignant melanoma.

PATIENTS AND METHODS

Patients entered in this study had histologically confirmed advanced malignant melanoma. Extent of disease was determined by physical examination, chest x-ray and radionuclide or computerized tomographic scanning when clinically indicated. Eligibility criteria included bidimensionally measurable disease, a wbc count > 4000/mm^3, a platelet count > 100,000/mm^3, a performance status of ≥ 60% (Karnofsky scale), serum bilirubin < 2 mg/dl, serum creatinine < 1.5 mg/ml, and no radiotherapy or chemotherapy for 4 weeks prior to starting treatment. Patients who had active cardiac disease or had received mediastinal irradiation were excluded from this trial. Signed informed consent was obtained from all patients.

4′-Epi-doxorubicin was administered as a slow iv infusion over 10–15 minutes every 3 weeks. For most patients the initial dose was 85 mg/m^2; however, seven patients with hepatosplenomegaly (but serum bilirubin concentrations < 2 mg/dl) were started at doses of 40–75 mg/m^2. Cbcs were obtained between Days 10 and 14 after each dose. In the absence of demonstrable myelosuppression, the dose was increased by 15 mg/m^2 on each subsequent cycle. One dose of the drug with evidence of myelosuppression was required for an individual patient's trial to be considered adequate.

Standard criteria of response were employed. Partial remission was defined as a ≥ 50% reduction in the sum of the products of the two greatest perpendicular diameters of all measurable lesions for at least 1 month. Minor response was defined as a reduction in measurable lesions of at least 25%, but less than that required for a partial remission. Disease progression was defined as an increase in the size of any lesion or the appearance of new lesions.

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RESULTS

Thirty-five patients were entered in this trial; 30 are evaluable for therapeutic effect. Three patients died of progressive melanoma within 4 weeks of beginning therapy and two patients were lost to follow-up. Of the 30 evaluable patients, 19 were men and 11 were women. The median age was 47 years (range, 28–67), and the median performance status was 80% (range, 60%–90%). In 13 patients the only sites of disease were skin or lymph nodes. Nine patients had lung involvement with or without cutaneous or nodal disease, six had liver metastases, one had a large mediastinal mass, and one had a large pelvic mass. Only three patients had received prior systemic chemotherapy. Additionally, three patients had been previously injected with intralesional BCG and three patients had received radiotherapy; previously injected or irradiated sites were not used as indicator lesions.

Partial remissions were noted in three patients (9%). None of these patients had received prior chemotherapy and each had a performance status of $\geq$ 70%. These responses occurred in patients with pelvic mass (duration, 6 months), lung nodules (duration, 6 months), and subcutaneous nodules (duration, 4 months). In addition, minor responses lasting 3, 4, and 2 months were noted in one patient each with cervical lymphadenopathy, lung nodules, and liver metastases, respectively.

Ninety-four doses of 4′-epi-doxorubicin were administered to the 30 evaluable patients. The total dose per patient ranged from 85 to 650 mg/m^2. The most prominent toxic effect in this study was myelosuppression, which was greatest 10–14 days after administration of the drug and was reversible by Days 21–28. Cumulative myelosuppression was not observed in patients treated with multiple doses. The median wbc count nadir for all patients was 3500/mm^3 (range, 900–10,000), and the median platelet count nadir was 247,000/mm^3 (range, 82,000–516,000). There were two episodes of sepsis at the time of nadir counts, but no deaths occurred as a result of treatment with 4′-epi-doxorubicin. No patient developed drug-induced anemia requiring transfusion.

Although approximately 20% of the patients reported mild nausea, only 5% had vomiting following treatment. One-third of the patients developed some degree of alopecia. Mucositis, phlebitis, and clinically evident cardiac toxicity were not observed.

DISCUSSION

In this trial, 4′-epi-doxorubicin was given iv once every 3 weeks to patients with advanced malignant melanoma. The patients had good performance status and most had not received prior systemic therapy. Although myelosuppressive doses were used, treatment resulted in a minimum of patient discomfort.

The 9% major objective response rate (95% confidence limits, 3%–23%) observed in this group of patients indicates that as a single agent, and when used on this schedule, 4′-epi-doxorubicin has limited therapeutic activity in the treatment of malignant melanoma.

REFERENCES

1. COMIS RL, and CARTER SK. Integration of chemotherapy into combined modality therapy of solid tumors IV: malignant melanoma. Cancer Treat Rev 1:285–304, 1974.
2. BLUM RH, and CARTER SK. Adriamycin: a new anticancer drug with significant clinical activity. Ann Intern Med 80:249–259, 1974.
3. O'BRYAN RM, LUCE JK, TALLEY RW, ET AL. Phase II evaluation of adriamycin in human neoplasia. Cancer 32:1–8, 1973.
4. EORTC CLINICAL SCREENING GROUP. Preliminary results of a phase II trial on solid tumors of detorubicin, a new anthracycline. Cancer Clin Trials 3:115–120, 1980.
5. BONFANTE V, BONADONNA G, VILLANI F, ET AL. Preliminary phase I study of 4′-epi-adriamycin. Cancer Treat Rep 63:915–918, 1979.
6. SCHAUER P, WITTES RE, GRALLA RJ, ET AL. A phase I trial of 4′-epi-adriamycin. Cancer Clin Trials 4:433–437, 1981.

Phase I–II Study of the Continuous Infusion of Doxorubicin in the Treatment of Non-Small Cell Lung Cancer[1,2]

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To circumvent or at least limit the cardiotoxicity of doxorubicin, a number of approaches have been attempted. These include the use of cardioprotective agents, toxic anthracycline analogs, and schedule alterations. To test the concept of schedule alteration we embarked on a phase I–II study of continuous infusions of doxorubicin, initially by 24-hour and subsequently by 6-hour infusions. The two schedules were chosen to evaluate the feasibility of each for future studies of cardiotoxicity.

METHODS

Patients were required to have histologically proven non-small cell carcinoma of the lung with an expected survival of at least 6 weeks and an Eastern Cooperative Oncology Group (ECOG) performance status of 3. Toxicity and performance status were evaluated on the basis of ECOG criteria (1). The patients could have had prior chemotherapy or radiotherapy but were required to have adequate marrow (wbc count $\geq 3.0 \times 10^3/mm^3$ and platelet count $\geq 100 \times 10^3/mm^3$) and liver (total bilirubin ≥ 3.0 mg/100 ml) functions.

Patients were divided into two groups: (*a*) those treated with a continuous 24-hour infusion, and (*b*) those treated with a continuous 6-hour infusion. The starting dose of doxorubicin was 60 mg/m^2, with escalations to 75 and 90 mg/m^2 according to nadir wbc and platelet counts, and treatment was repeated every 3 weeks. Clinical and laboratory evaluations were performed weekly. Doxorubicin was diluted in 500 ml of 5% dextrose in water and infused via a central catheter inserted into the femoral, the brachial, or the subclavian vein. The catheters were removed at the end of each infusion. Treatment parameters are listed in table 1.

RESULTS

Thirty patients were entered in this study. Nineteen patients were treated with a 24-hour infusion and 11 were treated with a 6-hour infusion. A total of 84 infusions were administered (25 6-hour infusions and 59 24-hour infusions). Responses to treatment were assessed in 22 patients (16 previously untreated) with evaluable disease, while toxicity was assessed in all 29 patients. Only one patient achieved a partial response. Toxicity was mainly hematologic (table 1), with a limiting dose of 75 mg/m^2 in the 6-hour trial and of 90 mg/m^2 in the 24-hour trial. This difference is probably explained by the larger number of pretreated patients in the 6-hour trial. Acute systemic toxicity was generally mild, with nausea and vomiting occurring in only 13 of the 29 patients; when it occurred, it was limited to the last hour of therapy and the 2–3 hours following it. Mild mucositis (grade ≤ 2) was seen in only two patients. Femoral thrombophlebitis occurred in one patient and resolved with heparin therapy.

DISCUSSION

This study demonstrates the feasibility of administering doxorubicin as an infusion through central venous lines including the femoral route. The use of this latter technique has been described in detail (2). Patients tolerated the infusions with negligible complication rates, the sole disadvantage being the obligatory admission to hospital. The systemic toxicity of doxorubicin given by this route and schedule did not appear to be increased and some toxic effects, especially nausea and vomiting, did appear to be lessened.

The efficacy of doxorubicin in non-small cell lung cancer was low and was similar to that reported in other studies (3). This result is likely attributed to the low intrinsic activity against lung cancer and not to the route and schedule of doxorubicin. The 6-hour schedule proved to be more feasible in a busy hospital. Whether an infusion schedule such as this is cardioprotective remains to be answered and is the subject of current study (4). Studies reported by others using endomyocardial biopsy as

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TABLE 1.—Patient characteristics and hematologic toxicity

Characteristic	Doxorubicin infusion	
	24 hrs	6 hrs
No. of patients	19	11
Median age in yrs (range)	62 (44-78)	60 (49-85)
Median performance status (ECOG)	2 (0-3)	2 (0-3)
No. of infusions	59	25
Route of infusions		
Femoral	23	23
Brachial	32	0
Subclavian	4	2
Median No. of infusions/patient	2	2
Median dose of doxorubicin (range), mg/m^2	75 (60-90)	60 (60-75)
Median cumulative dose of doxorubicin (range), mg/m^2	135 (60-820)	128 (60-360)
No. of patients with prior treatment	3 (16%)	5 (50%)
Histologic type (No. of patients)		
Squamous cell carcinoma	10	5
Adenocarcinoma	6	3
Undifferentiated carcinoma	3	2
Hematologic toxicity *		
60 mg/m^2		
Wbc count	3.5 (2.1-9.2)	2.4 (0.4-5.6)
Platelet count	2.0 (120-410)	182 (61-528)
75 mg/m^2		
Wbc count	3.0 (0.7-13.5)	2.0 (0.9-3.0)
Platelet count	220 (45-710)	245 (89-498)
90 mg/m^2		
Wbc count	2.7 (0.3-7.0)	
Platelet count	188 (75-413)	

* Median count (range), $\times 10^3$ cells/mm^3.

the indicator of cardiac toxicity suggest that infusion schedules protect against toxicity (5). If this is so, then the question of duration and method of administration of doxorubicin becomes important. If high peak levels are important in the development of cardiotoxicity, then one would believe that the longer the duration of the infusion, the lower the levels and the less the risk of cardiotoxicity. These questions remain to be answered by further trials with various infusion schedules of anthracyclines.

REFERENCES

1. OKEN M, CREECH R, TORMEY D, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Cancer Clin Trials, 1983. In press.
2. NIDUS BD, SPEYER JL, BOTTINO J, ET AL. Repeated femoral vein cannulation for administration of chemotherapeutic agents. Cancer Treat Rep 67:185-186, 1983.
3. CARTER SK. Adriamycin—a review. JNCI 55:1265-1273, 1975.
4. GREEN MD, SPEYER JL, and MUGGIA FM. Anthracycline cardiotoxicity: a perspective from current clinical studies. Cancer Treat Symp 1:71-73, 1983.
5. LEGHA SS, BENJAMIN RS, MACKAY B, ET AL. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 96:133-139, 1982.

Treatment of Advanced Soft Tissue Sarcomas With Low-Dose Methotrexate: A Phase II Trial by the European Organization for Research on Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group[1]

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Only a few drugs have shown some activity against advanced soft tissue sarcomas of the adult refractory to doxorubicin-containing combinations (1,2). Wiltshaw et al (3) observed a 25% remission rate in 52 previously untreated patients who received methotrexate in different doses and schedules. This study was conducted to further evaluate the activity of low-dose methotrexate in adult patients with advanced soft tissue sarcomas resistant to conventional therapy.

PATIENTS AND METHODS

Thirty-seven patients with measurable soft tissue sarcoma resistant to cyclophosphamide, vincristine, doxorubicin, and dacarbazine (CYVADIC) were entered in the study. Eight patients were not evaluable (forms never submitted, four; ineligible, two; and treatment never started, two). Patient characteristics are shown in table 1.

TABLE 1.—Patient characteristics and results

	No. of patients
Total evaluable	21
Sex	
Male	12
Female	9
Mean age in yrs (range): 40 (16–67)	
Disease status	
Local recurrence only	1
Local recurrence and metastatic	13
Metastatic only	7
Treatment	
Mean dose (range): 45 mg/m^2 (10–100)	
Mean No. of cycles (range): 7 (1–17)	
Objective remissions	0

Methotrexate was given weekly by iv push at an initial dose of 40 mg/m^2; the dose was increased weekly by 10 mg/m^2. Escalation was stopped in the presence of moderate stomatitis, wbc counts $\leq$ 3000/mm^3, or platelet counts $\leq$ 75,000/mm^3, and treatment was continued at the previous dose level.

RESULTS

No objective remissions occurred among 21 evaluable patients. Two patients who refused therapy after two cycles and six patients with progressive disease before the 6th week were considered only for toxicity analysis. Toxic effects are described in table 2.

DISCUSSION

Two different studies have explored the activity of high-dose methotrexate in adult soft tissue sarcomas. Karakousis et al (4) reported one partial remission among 18 patients who had progressed on the CYVADIC regimen, while Isacoff et al (5) observed five objective remissions among 11 patients with metastasic disease resistant to different combinations which included doxorubicin.

TABLE 2.—Toxic effects

Toxic effect	% cycles (% patients)
Wbc count (cells/mm^3)	
2000–3000	10 (41)
< 2000	3 (20)
Platelet count (cells/mm^3)	
25,000–50,000	2 (14)
Stomatitis	
Moderate	11 (38)
Severe	5 (27)

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Our study illustrates the problems that may be encountered in evaluating agents in patients who have previously received multiple drugs. The results of this study do not exclude the possibility that methotrexate is active as first-line therapy in this group of tumors.

REFERENCES

1. PINEDO HM, and KENIS Y. Chemotherapy of advanced soft tissue sarcomas in adults. Cancer Treat Rev 4:67–86, 1977.
2. STEWART DJ, BENJAMIN RS, BAKER LH, ET AL. New drugs for the treatment of soft tissue sarcomas. *In* Therapeutic Progress in Ovarian Cancer, Testicular Cancer and the Sarcomas (van Oosterom AT, Muggia FM, and Cleton FJ, eds). The Hague, Martinus Nijhoff Publishers, 1980, pp 453–479.
3. WILTSHAW E, HARMER CL, and MCKINNA JA. Soft tissue sarcomas: treatment of advanced disease in the Royal Marsden Hospital. *In* Therapeutic Progress in Ovarian Cancer, Testicular Cancer and the Sarcomas (van Oosterom AT, Muggia FM, and Cleton FJ, eds). The Hague, Martinus Nijhoff Publishers, 1980, pp 413–423.
4. KARAKOUSIS CP, RAO U, and CARLSON M. High-dose methotrexate as secondary chemotherapy in metastatic soft-tissue sarcomas. Cancer 46:1345–1348, 1980.
5. ISACOFF WH, EILBER F, TABBARAH H, ET AL. Phase II clinical trial with high-dose methotrexate therapy and citrovorum factor rescue. Cancer Treat Rep 62:1295–1304, 1978.

Chlorozotocin: Phase II Evaluation in Patients With Myeloma[1,2]

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For the Cancer and Leukemia Group B

Chlorozotocin, a chloroethylnitrosourea with demonstrated antitumor activity against L1210 leukemia, has shown less marrow toxicity at equimolar plasma concentrations which produced toxicity with carmustine or lomustine (1). The Cancer and Leukemia Group B (CALGB) studied this agent in a phase II trial in patients with refractory myeloma.

Sixty-two patients were initially entered in the study. Fifty-five patients were evaluable; seven were excluded from all analysis (ineligible, two; incomplete data, five). Patient characteristics are shown in table 1. Most patients had stage III disease, had previously been treated with an average of three chemotherapeutic agents (some with nitrosoureas), and met Myeloma Task Force criteria

TABLE 1.—Patient characteristics, treatment schedules, hematologic toxicity, and response to treatment*

Characteristic	Total patients	Patients with prior nitrosourea therapy	Patients without prior nitrosourea therapy
Total evaluable	(55)	(25)	(30)
Mean age in yrs	61.5	60.6	62.5
Male/female	(50/50)	(40/60)	(60/40)
Median No. of mos from diagnosis to start of protocol		21	26
Initial performance status†			
0	13 (7)	9 (2)	17 (5)
1	24 (13)	17 (4)	30 (9)
2	24 (13)	17 (4)	30 (9)
3	29 (16)	48 (11)	17 (5)
4	11 (6)	13 (3)	10 (3)
Dose of chlorozotocin (120 mg/m^2)			
Full dose	31	32	30
Reduced up to 50%	47	40	53
Reduced > 50%	22	28	17
Schedule (q6wks)			
Given on schedule	45	38	47
Extended intervals	39	38	37
Shortened intervals	13	15	11
Skipped doses	6	7	5
Duration of therapy (wks)			
< 6	33	52	17
6 to < 12	24	24	23
12 to < 18	14	4	23
18 to < 24	18	12	23
> 24	11	8	13

continued

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TABLE 1.—*Continued*

Characteristic	Total patients	Patients with prior nitrosourea therapy	Patients without prior nitrosourea therapy
Hematologic toxicity‡			
0	80/30	76/29	81/42
1	9/30	6/35	11/19
2	9/28	18/29	4/23
3	2/12	0/6	4/15
4	0/0	0/0	0/0
Response			
Objective	4 (2)	0 (0)	7 (2) [124]§
Improved	7 (4)	4 (1) [181]§	10 (3) [131]§
Stable disease	9 (5)	4 (1)	13 (4)
Disease progression	80 (44)	92 (23)	70 (21)
Died on study (without response)	33 (18)	44 (11)	23 (7)
Off study alive (with or without response)	67 (37)	56 (14)	77 (23)

*Unless otherwise specified, values = % of patients (No. of patients).

†Initial performance status as defined in the CALGB Myeloma Master Protocol. Grade 0 = patient's activities normal; grade 1 = mildly symptomatic but ambulatory; grade 2 = moderately severe symptoms, in bed < 50% of waking time; grade 3 = severe symptoms, in bed > 50% of waking time; and grade 4 = completely bedridden.

‡Values = % with leukopenia/% with thrombocytopenia. Grades are defined as follows ($\times 10^3$ cells/mm^3): grade 0 = wbc count > 5, platelet count > 100; grade 1 = wbc count of 4–5, platelet count of 75–100; grade 2 = wbc count of 3–4, platelet count of 50–75; grade 3 = wbc count of 2–3, platelet count of 30–50; and grade 4 = wbc count < 2, platelet count < 30.

§Mean duration of response in days.

for diagnosis. They were treated with chlorozotocin (120 mg/m^2) every 6 weeks (2,3). Following the initiation of treatment, dosages were modified for excessive hematologic toxicity.[5] Patients were monitored for evidence of disease improvement or progression (myeloma protein, cbc, serum calcium, and bone lesions) every 6 weeks as well as for hepatic, renal, and pulmonary toxicity.

Objective responses (a 50% reduction of myeloma protein) were achieved in two patients. Four patients had reductions of 25%–49% and were regarded as improved. Five patients appeared to have stable or nonprogressive disease. Forty-four patients showed evidence of disease progression. One of the 25 patients with previous exposure to a nitrosourea was considered improved; all others in this group were nonresponders. Hematologic toxicity was the only dose-limiting factor; moderate thrombocytopenia occurred, particularly in patients with previous nitrosourea exposure. There was no evidence of renal, pulmonary, or hepatic toxicity.

Disease response to chlorozotocin was limited. When the analysis was limited only to the 30 patients without prior nitrosourea therapy, the objective response rate was 7%. This was less than the 17% response rate reported for patients failing initial alkylating-agent therapy who received the combination of prednisone, cyclophosphamide, and carmustine in a previous CALGB protocol (4).

There were only six patients with prior nitrosourea therapy with good performance scores; therefore, the question of cross-resistance between chlorozotocin and other nitrosoureas cannot be addressed satisfactorily. Because of the low-level activity observed with chlorozotocin alone in relapsed myeloma patients, it cannot be recommended for use in these patients.

[5]Dose was reduced as percentage of full dose, according to toxicity grade (0 = 100%, 1 = 75%, 2 = 50%, 3 = 25%, and 4 = 0%). *See* table 1.

REFERENCES

1. ANDERSON T, MCMENAMIN M, and SCHEIN P. Chlorozotocin 2-(3-(2-chloroethyl)-3-nitrosourea, an antitumor agent with modified bone marrow toxicity. Cancer Res 35:761–765, 1975.
2. DURIE BGM, and SALMON SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 36:842–854, 1975.
3. CHRONIC LEUKEMIA-MYELOMA TASK FORCE. Proposed guidelines for protocol studies. Cancer Chemother Rep Part 3, vol 4(1):145–158, 1973.
4. KYLE RA, GAILANI S, SELIGMAN BR, ET AL. Multiple myeloma resistant to melphalan: treatment with cyclophosphamide, prednisone, and BCNU. Cancer Treat Rep 63:1265–1269, 1979.

Phase II Trial of Bisantrene in Advanced Epidermoid Carcinoma of the Head and Neck[1,2]

Arlene A. Forastiere,[3,*] Sidney M. Crain,[3] Carlos Garbino,[4] Stuart J. Tipping,[3] David J. Perry,[5] Helmut Kasdorf,[4] and David A. Van Echo[3]

Bisantrene is an anthracenedicarboxaldehyde hydrazone with a wide spectrum of antitumor activity in animal models, similar to doxorubicin, but without evidence of dose-limiting cardiotoxicity or alopecia (1). In phase I–II testing, activity has been reported in renal cell, breast, and hepatocellular carcinomas. The dose-limiting toxic effect was myelosuppression; nausea, vomiting, phlebitis, hypotension, and mild alopecia were other potential toxic effects (2–4). This study evaluated the activity of bisantrene in head and neck cancer.

METHODS AND MATERIALS

Twenty-six patients with histologically confirmed epidermoid carcinoma of the head and neck were entered in the study. All patients had measurable disease, a Karnofsky performance score of at least 50%, an expected survival of at least 8 weeks, wbc count $\geq 4000/\mu l$, platelet count $\geq 125{,}000/\mu l$, serum creatinine < 2.0 mg/dl, and serum bilirubin < 2.0 mg/dl. A chest roentgenogram, 12-lead electrocardiogram, serum electrolytes, and liver function tests were required prior to entry in the study. Patients with an unstable cardiac status were excluded.

The initial dose of bisantrene was 260 mg/m^2 diluted to a volume of 500 ml of 5% dextrose in water (USP) administered by peripheral iv infusion over no less than 60 minutes. Treatment was repeated every 3 weeks. In the absence of toxicity, one dose escalation to 280 mg/m^2 was allowed. If the wbc count nadir was $< 1500/\mu l$ or the platelet count nadir was $< 50{,}000/\mu l$, the dose was reduced to 220 mg/m^2. Cbc and platelet count were repeated weekly and measurable disease parameters were assessed every 3 weeks. An adequate trial required two cycles or rapid progression of disease after one cycle at a dose producing a wbc count nadir $< 3000/\mu l$. Standard response criteria defined in previous trials were used (5).

RESULTS

Patient characteristics are shown in table 1. Seven patients had received prior non-doxorubicin-containing chemotherapy for recurrent disease. Six patients presenting with bulky, unresectable stage IV disease were treated with bisantrene prior to irradiation. Twenty-one patients (five previously untreated) were evaluable for response. Three patients were lost to follow-up and there were two early deaths prior to response evaluation.

One partial response was observed in one patient with a $T_4N_3M_0$ carcinoma of the pyriform sinus who had received no prior therapy. After three courses of bisantrene, the patient was treated with radiation therapy with complete regression of tumor at the primary site. Two other patients demonstrated tumor regression, although less than that required for a partial response: one patient had recurrent disease, while the other had received no prior therapy and was referred for radiation therapy after two cycles. Myelosuppression, predominantly leukopenia, was the major toxic effect observed. There was one death from pseudomonas septicemia in a patient with a wbc count nadir of $300/\mu l$. Gastrointestinal toxicity occurred rarely; however, all patients were premedicated with a variety of antiemetic regimens. Transient hypotension, responding to fluids and reduced rate of drug infusion, occurred in four patients; phlebitis occurred in two. Treatment was stopped in one patient with stable disease after four cycles because of bilateral upper extremity edema.

DISCUSSION

Only one major response was observed in this phase II trial of bisantrene for advanced epidermoid carcinoma of the head and neck. It is of interest that this occurred in a

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TABLE 1.—Patient characteristics and treatment results

	No. of patients
Total	26
Median age in yrs (range): 57 (38–83)	
Median performance score (range): 70 (50–90)	
Males/females	21/5
Prior treatment	
Radiation therapy only	3
Surgery and radiation therapy	10
Radiation therapy and chemotherapy	1
Surgery, radiation therapy, and chemotherapy	6
No prior treatment	6
Primary site	
Oral cavity	2
Oropharynx	15
Larynx	5
Pyriform sinus	3
Nasopharynx	1
Sites of measurable disease	
Primary	18
Regional nodes	12
Skin	1
Lung	5
Courses of bisantrene	
1	14
2	7
3	3
4	2
Response (21 evaluable patients)	
Partial	1
Minor	2
Stable disease	2
Disease progression	16
Toxic effects	
Hematologic (cells/μl)*	
Wbc count	
3000–3999	6
2000–2999	6
1000–1999	1
< 1000	3
Platelet count	
50,000–100,000	1
< 50,000	0
Nausea and vomiting	2
Hypotension	4
Phlebitis	2

* Lowest count recorded.

patient who had bulky, previously untreated disease. Although most evaluable patients (16 of 21) had been previously treated with surgery and/or radiotherapy, only seven had received prior chemotherapy; the median performance status was 70%. Based on these results we conclude that bisantrene at this dose and schedule is not a useful agent in the treatment of recurrent epidermoid carcinoma of the head and neck.

REFERENCES

1. CITARELLA RV, WALLACE RE, MURDOCK KC, ET AL. Activity of a novel anthracenyl bishydrazone, 9,10-anthracenedicarboxaldehyde bis (4,5-dihydro-1*H*-imidazol-2yl) hydrazone) dihydrochloride, against experimental tumors in mice. Cancer Res 42:440–444, 1982.
2. VON HOFF DD, MYERS JW, ET AL. Phase I clinical investigation of 9-10 anthracenedicarboxaldehyde bis ((4,5-dihydro-1*H*-imidazol-12-yl) hydrazone) dihydrochloride (CL 216, 942). Cancer Res 41:3118–3121, 1981.
3. OSBORNE CK, VON HOFF DD, and SANDBACH J. Activity of bisantrene in a phase II study in advanced breast cancer. Proc ASCO 1:87, 1982.
4. MYERS JW, VON HOFF DD, COLTMAN CA, JR, ET AL. Phase II evaluation of bisantrene in patients with renal cell carcinoma. Cancer Treat Rep 66:1869–1871, 1982.
5. FORASTIERE AA, YOUNG CW, and WITTES RE. A phase II trial of *m*-AMSA in head and neck cancer. Cancer Chemother Pharmacol 6:145–146, 1981.

Phase II Trial of 4-Demethoxydaunorubicin in Advanced Colorectal Carcinoma[1,2]

Harry D. Harper, Nancy E. Kemeny,* Tauseef Ahmed, Edgar Cheng, Francis Arena, Peter Sordillo, and Leslie Faye[3]

4-Demethoxydaunorubicin (DMDR) is a derivative of daunorubicin which lacks the methoxy group in position four of the aglycone (1). DMDR was extended into clinical trial because of its activity against a number of experimental tumor systems, eg, L1210 leukemia, Gross leukemia, and Sarcoma 180 (1,2), and because it is significantly less cardiotoxic than daunorubicin or doxorubicin when administered in animal model systems (3).

In phase I evaluation, the dose-limiting toxic effect of DMDR was myelosuppression; an iv dose of 12.5 mg/m^2 given every 3 weeks was suggested for use in phase II trials (4,5). In this communication, we report the results of a phase II study of iv DMDR in patients with advanced measurable colorectal carcinoma.

MATERIALS AND METHODS

DMDR was supplied by Farmitalia Carlo Erba, Milan, Italy; the drug was given iv at a starting dose of 12.5 mg/m^2 every 3 weeks.

Patients were eligible for this study if they had measurable, inoperable, histologically confirmed colorectal carcinoma. Patients were required to have a Karnofsky performance status > 50%, a serum creatinine < 1.5 mg/dl, a serum bilirubin < 1.5 mg/dl, a wbc count > 3500/mm^3, and a platelet count > 150,000/mm^3, and had to be capable of giving informed consent for entry in the study.

After each dose, cbcs were monitored on a weekly basis. Subsequent drug doses were escalated by 20% (to 15 mg/m^2) if no myelosuppression was seen.

A complete response was defined as the disappearance of all clinical evidence of tumor; a partial response was defined as a > 50% reduction in the summed products of the perpendicular diameters of bidimensionally measurable lesions. Stabilization of disease was defined as a < 25% change in previously progressing measurable disease > 3 months. Progression of disease was defined as a > 25% change in measurable disease parameters or the appearance of new lesions. At least two doses of DMDR had to be administered for the trial to be considered adequate.

Patient Characteristics

Forty-three patients were entered in the study, with a median age of 61 years (range, 36–80); 26 patients were male and 17 were female. The median Karnofsky performance status was 70% (range, 60%–90%). The primary tumor was in the colon in 36 patients and in the rectum in seven. Fourteen patients had received no prior chemotherapy. Of the remaining patients, the median number of prior treatment regimens was one (range, one to four). The indicator lesions were hepatic in 33 patients (77%), pulmonary in five (12%), abdominal masses in four (7%), and cervical lymph nodes in one.

The dose administered ranged from 7.5 to 17.5 mg/m^2; the median number of doses was two (range, one to seven).

RESULTS

Thirty-five of the 43 patients had an adequate trial of DMDR. Seven of the eight inevaluable patients received only one dose because of rapid progression of disease and/or clinical deterioration during the brief interval between planned doses. One patient refused further therapy due to intolerable nausea.

One patient with cervical lymphadenopathy from a colon tumor had a partial response lasting 2 months. Two patients had stable disease for 3–5 months.

Toxicity

The median wbc count nadir was 2900/mm^3 (range, 200–11,100), with 18 patients having a wbc count

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[3]Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

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< 2000/mm^3 at some point during their treatment. The median platelet count nadir was 199,000/mm^3 (range, 24,000–558,000), with 11 patients having a platelet count nadir < 100,000/mm^3. Two patients required hospitalization for drug-related neutropenia and fever. Mild nausea was seen in 17 patients and vomiting was seen in ten. No alopecia or mucositis was noted.

DISCUSSION

Forty-three patients with metastatic colorectal carcinoma were treated with DMDR at a dose of 12.5 mg/m^2 iv every 3 weeks. The drug was well-tolerated; myelosuppression was the most common toxic effect. One partial response occurred among 14 previously untreated patients, for a response rate of 7% (95% confidence interval, 0%–21%). No responses occurred among the 21 evaluable previously treated patients. The response rate for the entire study (one response among 35 evaluable patients) was 2.9% (95% confidence interval, 0%–8%). Based on these results, we conclude that DMDR at this dose and schedule has minimal activity as a single agent in the treatment of colorectal carcinoma.

REFERENCES

1. CASAZZA AM, PRATESI G, GIULIANI F, ET AL. Anti-leukemic activity of 4-demethoxydaunorubicin in mice. Tumori 66:549–565, 1980.
2. ARCAMORE F, BERNARDI L, GIARDINO P, ET AL. Synthesis and anti-tumor activity of 4-demethoxydaunorubicin, 4-demethoxy-7,9-diepi-daunorubicin, and their β anomers. Cancer Treat Rep 60:829–834, 1976.
3. CASAZZA AM, DIMARCO A, BONADONNA G, ET AL. Effects of modification in position 4 of the chromophore or in position 4′ of the amino-sugar on the anti-tumor activity and toxicity of daunorubicin or doxorubicin. *In* Anthracyclines: Current Status and New Developments (Crooke ST, and Reich SD, eds). New York, Academic Press, 1980, pp 403–430.
4. BERMAN E, WITTES R, LEYLAND-JONES B, ET AL. 4-Demethoxydaunorubicin: phase I clinical and pharmacological studies in patients with advanced cancer. Cancer Res, in press.
5. KAPLAN S, MARTINI A, VARINI M, ET AL. Phase I trial of 4-demethoxydaunorubicin with single iv doses. Eur J Cancer Clin Oncol 12:1303–1306, 1982.

First European Conference on Advances in Antitumor Agents

Milan, Italy, May 30–June 1, 1984

This conference was organized by the Associazione Farmaceutici Industria and Consiglio Nazionale delle Ricerche-C.N.R., Progetto Finalizzato Chimica Fine e Secondaria. The meeting is to be restricted to basic sciences (chemistry and biology) and will focus on critical re-examination of present approaches and proposals of new ones. The following topics will be dealt with: new antitumor agents of natural and synthetic origin; molecular interactions and metabolism; evaluation of screening programs; and peptides and hormones.

The text of the invited lectures will not be published in the form of symposium-proceedings in order to allow free and informal discussion and protect individual rights. A limited number of short papers will be considered for oral communication. In addition, a poster session has been planned. The official languages of the conference will be English and Italian and simultaneous translation in these languages will be provided.

For more information, contact: Conference Secretariat, A. F. I., Via S. Tecla, 4-20122 Milan, Italy (telephone: Farmitalia-Carlo Erba, Milan, Italy).

Rational Therapeutics: A Seminar in Oncology

Duarte, California, June 2–3, 1984

This ninth annual symposium is sponsored by the Department of Pharmacy Services, City of Hope National Medical Center and will be held at the Anaheim Marriott Hotel in Anaheim, California. It is designed to provide members of the health care team with current and clinically useful information in the management of the cancer patient including the drug selection process, patient evaluation, and monitoring techniques.

This year's program will feature topics on: Empiric Antibiotic Therapy in the Immunocompromised Host, Nutritional Support in the Cancer Patient, VP-16 Chemotherapy, Cisplatin: 5 Years Later, Pharmacokinetic Studies of Anti-Cancer Therapy, Oncologic Emergencies, and Influence of Nutrition on the Development of Cancer. In addition, the symposium will have several clinical tutorials available covering specific areas.

The cost of this seminar is $120. Twelve hours of accreditation from either ACPE, California State Board of Pharmacy, or the California State Board of Nursing have been applied for. Physicians attending this course may report up to 12 hours of formal category I credit towards the California Medical Association Certificate of Continuing Medical Education.

For further information, contact: Debra Chung, Pharm D, Program Chairperson, Department of Pharmacy Services, City of Hope National Medical Center, 1500 E Duarte Rd, Duarte, CA 91010 (telephone: 818-359-8111 x 2247).

Koloman Laki Memorial Symposium on Haemostasis and Cancer

Budapest, Hungary, July 9–11, 1984

In memory of the late Dr. Koloman Laki, codiscoverer of Factor XIII and author of many highly valuable contributions to the fields of haemostasis and cancer, an International Symposium on Haemostasis and Cancer, jointly organized by the National Foundation for Cancer Research, and the Hungarian Society on Haematology, Haemostasis Section, will be held at the Hotel Hyatt Atrium in Budapest.

The symposium is open to scientists interested in various aspects of the relationship of haemostatic systems and malignant cell proliferation and metastasis. Lectures will focus on procoagulant materials produced by cancer cells, the role of fibrinogen-fibrin-Factor XIII in cancer cell proliferation, the effect of anticoagulant and antiplatelet drugs on malignant tumors, and on platelet-malignant cell interaction. Additionally, there will be posters and oral presentations. The number of the participants is limited to 100. The Congress language will be English.

For further information, contact: Dr. L. Muszbek, University School of Medicine, Department of Clinical Chemistry, PO Box 40, Debrecen Hungary H-4012 (telephone: /36/-52-17631, telex: 72-411 dote-h).

Fellowship in Medical Oncology, Hematology, or Combined Oncology-Hematology

July 1984 and July 1985

Applications are now being accepted for a fellowship program involving major teaching hospitals of Brown University's Program in Medicine, beginning July 1984 and July 1985. Clinical activities and opportunities for research are offered to qualified candidates with two years of approved medical residency and ABIM eligibility.

For further information, contact: F. J. Cummings, MD, Director, Division of Medical Oncology, or Manfred Steiner, MD, Director, Division of Hematology, Brown University, Box G, Providence, RI 02912.

Treatment of Metastasis: Problems and Prospects

London, England, October 15-17, 1984

The conference, an international meeting to be held at the Strand Palace Hotel is open to all those who are interested, but early application is advised. Registration and abstract forms will be sent on request and should be returned with the registration fee of £50 (£30 for research students on grants) no later than June 30, 1984. The registration fee covers the provision of programs, abstracts, lunches, and refreshments throughout the meeting. The proceedings of the meeting will be published. All papers on the subject of the meeting for oral or poster presentation will be considered.

For further information, contact: K. Hellmann, DM, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, England.

Symposium on Cancer Chemosensitivity Assay

Long Beach, California, October 21-25, 1984

Long Beach Community Hospital's Llewellyn Bixby IV Hematology-Oncology Laboratory, under the direction of Nathaniel B. Kurnick, MD, is sponsoring this international symposium to be held at the Hyatt Regency Hotel.

The conference will provide a forum for the most active and pre-eminent investigators in the field of cancer chemosensitivity assay to share their knowledge, exchange ideas, and to discuss new techniques and methodologies. It is accredited by the California Medical Association for 19 hours of category one continuing education credit.

With emphasis on methodology and applicability, topics will include: Radio-nuclide methods, Clonogenic and other tissue culture methods, Animal models and xenografts, and other methods, such as histologic (including dye exclusion), metabolic, cytologic, and membrane integrity.

The symposium will be of interest to clinical oncologists, hematologists, and surgeons and will offer newer investigators the opportunity to meet with the established researchers from various parts of the world and to present papers, abstracts, or posters during the afternoon sessions.

The $350 per participant registration fee includes conference registration, all printed materials including agenda and abstracts, a reception cocktail party, and a gala dinner.

For further information, contact: Mrs. Mickles (telephone 213-498-1000, ext 3600).

Innovative Cancer Chemotherapy for Tomorrow

New York, New York, November 7-9, 1984

The sixth biennial symposium of the Chemotherapy Foundation will be held at the Barbizon Plaza Hotel under the auspices of the Division of Medical Oncology of the Department of Medicine, the Department of Neoplastic Diseases, and the Page and William Black Post-Graduate School of Medicine, the Mount Sinai School of Medicine (CUNY). Dr. Ezra M. Greenspan, Clinical Professor of Medicine

(Oncology), is chairman of the three day meeting which will also include recent developments in chemo-immunotherapy and biological response modifiers. Symposium registrants are eligible for approximately 20 AMA Category I credits.

For further information, contact: Director, the Page and William Black Post-Graduate School of Medicine, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029 (telephone: 212-650-6772).

Sixth International Symposium on Prevention and Detection of Cancer

Vienna, Austria, November 26-29, 1984

Sponsored by the International Society for Preventive Oncology, World Health Organization, Austrian Cancer Society-Austrian Cancer League, and Association of Clinical Scientists-USA, the program for this symposium includes overview lectures, panels, poster sessions, scientific exhibits, and special workshops designed for critical appraisal of current data. Discussions are to concentrate on actions to be taken and on the implementation of existing knowledge for effective cancer control by primary and secondary prevention. Reports are to present progress in multifactorial etiology of oncogenesis, molecular biology, identification of high-risk groups, tumor susceptibility, and clinical and laboratory manifestations of cancer including tumor markers. Participants include the clinician, epidemiologist, pathologist, experimental oncology, immunologist, socioeconomist, educator and members of the health care team.

Abstracts of presentations are invited by June 15, 1984, abstracts and the program will appear in *Cancer Detection and Prevention*, 1984, vol 7, No. 6. Awards for outstanding investigation(s) presented by ISPO. Accredited for 32 CME credit hours.

For further information, contact: Prevention and Detection of Cancer, AMEX POB #790459, Dallas, TX 75379 [telephone: 1-800-527-0297; outside Europe (usa-214)392-3663; in Europe (austria 43-222)52-0544].

Neuro-Oncology and Neuropathology Fellowships

University of California, San Francisco, July 1985 and 1986

The neuro-oncology fellowship emphasizes clinical training in adult and childhood primary tumor therapeutics and research training in the Brain Tumor Research Center laboratories. Applicants for this position will be expected to have a valid California medical license. The neuropathology fellowship emphasizes primary tumor pathology and offers research opportunities in the Brain Tumor Research Center and the Department of Pathology.

The University of California is an equal opportunity/affirmative action employer. Women and minorities are encouraged to apply.

For further information, contact: Victor Levin, MD, Brain Tumor Research Center, HSW-783, University of California Medical School, San Francisco, CA 94143 (telephone: 415-666-2905).

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